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71868 U.S. PTO
7/23/97
Submitted herewith for filing
the patent application of

Inventor(s): COPPENS, et al.

For: PROCESS FOR THE PREPARATION
OF MALTED CEREALS

Enclosed are:

(X) 5 sheet(s) of drawing(s) (X) Informal

() Assignment(s) of the invention to _____

(X) Patent Application.

(X) Unsigned Declaration and Power of Attorney.

(X) Preliminary Amendment.

Fee Calculation For Claims As Filed

a) Basic Fee					\$ 770.00
b) Independent Claims	<u>8</u>	-	<u>3</u>	=	<u>5</u> x \$ 80.00 = \$ <u>400.00</u>
c) Total Claims	<u>42</u>	-	<u>20</u>	=	<u>22</u> x \$ 22.00 = \$ <u>484.00</u>
d) Fee for Multiply Dependent Claims					\$260.00 \$ <u>260.00</u>
Total Filing Fee					\$ <u>1,914.00</u>

() Statement(s) of Status as Small Entity,
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FITCH, EVEN, TABIN & FLANNERY

By: Timothy E. Levstik
Timothy E. Levstik
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Coppen et al.)
Serial No.:) CERTIFICATE OF MAILING BY "EXPRESS MAIL"
Filed:) Express Mail mailing label number
Title: PROCESS FOR THE) EI 589 724 593 US
PREPARATION OF) Date of Deposit July 23, 1996
MALTED CEREALS)
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PRELIMINARY AMENDMENT

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Dear Sir:

Prior to the calculation of filing fees in the
above-identified application, please enter the following
Amendments:

IN THE SPECIFICATION:

Please amend the following as the first line of
text:

This application is a continuation-in-part of
International application PCT/BE96/00077 filed July 23, 1996
and designating the United States.

IN THE CLAIMS:

Claim 16, delete "to 15" and insert therefor --or
14--.

Claim 25, delete "22,".

Claim 32, after 27, delete "to" and substitute
therefor --, 28, 29, 30 or--.

Claim 34, after 31, delete "or Claim 32".

Claim 35, after 27, delete "to 35" and substitute
therefor --, 28, 29, 30, 31 or 33--.

Claim 36, after 27, delete "to 35" and substitute therefor -- , 28, 29, 30, 31 or 33--.

Claim 37, after 27, delete "to 36" and substitute therefor -- , 28, 29, 30, 31 or 33--.

Claim 40, after of, insert --claim-- and delete "any one of the claims".

Claim 40, after 27, delete "to 37".

Claim 41, after 38, delete "to 40" and substitute therefor --or 39--.

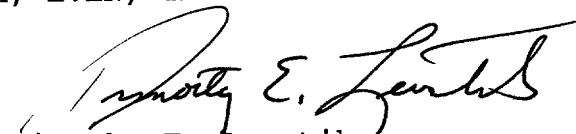
REMARKS

These amendments are requested to correct multiple dependencies.

Respectfully submitted,

FITCH, EVEN, TABIN & FLANNERY

By


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July 23, 1997

ATTORNEY DOCKET NO. 61944

Field of the Invention

The present invention is related to an improved process for the preparation of malted cereals, the improved malted cereals obtained and their use, especially in biotechnological processes for the preparation of beverages.

Technological Background of the Invention

Cereals, such as barley, wheat, rye, corn, oats, rice, millet, triticale, and sorghum, are used for the production of beverages. In most cases, they have been subjected to a malting process to take advantage of their increased enzymatic potential.

In traditional malting processes, the moisture content of cereals is raised either by immersion(s) and/or spraying(s), and the resulting high-moisture content cereal is allowed to germinate. After reaching the proper physiological condition, it is preferably submitted to (a) drying step(s). In what follows, the term steeping refers to the increase in moisture level, while the term germination is used in the way it is in plant physiology. The drying operations are referred to as kilning and the term malting involves all operations needed to convert barley (or other cereals) to barley malts (or other cereal malts).

The quality of the malt obtained is, to a large extent, determined by the presence of plant endogenous enzymes generated during the malting process. For instance, with cereals like barley used as a raw material for the malt production, the variety, the composition of the microbial flora and the environmental factors, such as agricultural practice, influence the quality of the malt. During cultivation and storage, cereals are contaminated with bacteria and

fungi. In the malting plant, neither the air, the water, nor the equipment are sterile, and the conditions of humidity, pH and temperature favor the growth of the microbial populations. To improve the quality of malted cereals, such as barley, enzymes have been added to the malted cereal.

The variable cereal quality and the lack of means to make up for deficiencies during the malting process result in variability in malt quality and enzymatic activity. In many instances, this has to do with an imbalance of specific enzymatic potential and insufficient cell wall degradation. Apart from this, problems with microbial safety can occur. As a consequence of the defects in malt, quality problems occur in the production of beer, such as a poor filtration of the wort.

State of the Art

During the malting of cereals, the microflora present on the cereals develops and the quality of the malt and beverages is influenced by the activity of the latter microorganisms.

In analogy with other biotechnological processes, there have been attempts to optimize malt quality aspects by the addition of starter cultures during the malting process (Boivin, P. & Malanda, M., Influence of Starter Cultures in Malting on the Microflora Development and Malt Quality, EBC, Proceedings of the 24th Congress, pp. 95-102 (1993); Haikara, A. et al., Lactic Starter Cultures in Malting - A Novel Solution to Gushing Problems, European Brewery Convention, Proceedings of the 24th Congress, pp. 163-172 (1993)).

Addition of spores of *Geotrichum candidum* to the steeping water results in the inhibition of the development of undesirable microorganisms and in a decrease of the filtration time of wort made of the obtained malt. Treatment with *Geotrichum candidum* also inhibits the formation of mycotoxins by *Fusarium* spp.

The influence of *Lactobacillus plantarum* and *Pediococcus pentosaceus* has been tested on the microflora during malting, and it has been found that

these cultures act as natural preservatives as they restrict the growth of *Fusarium* and prevent gushing.

The international patent application WO 94/29430 describes a process for improving the properties of malted cereals wherein starter cultures which comprise moulds, yeasts or bacteria are added prior and/or during malting of said cereals.

The preferred bacteria used are lactic acid producing bacteria, such as various *Lactobacilli*, e.g., *Lactobacillus casei*, *Lactobacillus casei* var *rhannosus*, *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis*, and bacteria of the genus *Pediococcus*, e.g., *Pediococcus acidilactici*.

Preferred moulds are moulds of the genus *Aspergillus* and *Geotrichum*, like *Geotrichum candidum*.

The international patent application WO94/16053 describes a process for treating cereals for inhibiting growth of unwanted microbial species by inoculating the cereals during the germination process with a lactic acid bacteria preparation or a preparation produced by lactic acid bacteria. The preferred bacteria are lactic acid bacteria belonging to genus *Lactococcus*, *Leuconostoc*, *Pediococcus* or *Lactobacillus*.

The British patent application GB-1211779 provides a method for the automatic control and regulation of a malting process. It enables one to determine the parameters necessary for a successful automatically controlled and regulated malting process.

In the proceedings of the European Brewery Convention, volume 16, 1977, pages 245 to 254, the influence of some fungi on malt quality is described, more specifically, contamination of barley malt with fungi which has led to gushing and other qualitative changes in the beer.

The German patent application DE-3028360 discloses a method to make malt out of corn.

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However, malt prepared according to the present invention is of better quality than that prepared according to WO 94/29430. This is exemplified by higher β -glucanase and xylanase activities, lower -glucan contents in malt and wort and improved European Brewery Convention analytical data.

Aims of the Invention

The present invention aims to provide an improved preparation process for malted cereals and improved malted cereals.

A main aim of the invention is to provide an improved preparation process for malted cereals and improved malted cereals in terms of brewing performances, especially malted cereals having an improved quality in terms of enzymatic potential and microbial safety.

Another aim is to provide a process and improved malted cereals which vary less in quality with the raw material used.

A further aim of the invention is to obtain malted cereals which improve the biotechnological production process of beverages and may improve the properties of the said obtained beverages.

Summary of the Invention

The present invention is directed to a process for the preparation of a malted cereal, the malted cereal which is the product of the process of the invention, and a combination of wetted or moistened cereal and activated spores which, when held for a sufficient time and temperature, provide a malted cereal product of enhanced enzymatic activity. The product of the invention has enhanced enzymatic activity of at least one enzyme, such as β -glucanase, xylanase, amylase, naturally occurring enzymes, and/or protease activity, over

malted cereal products which are similarly prepared with wetted cereal products with or without microorganisms.

The process of the invention utilizes activated spores from microorganisms such as bacteria or moulds. The process generally comprises combining water, the cereal and activated spores and holding the combination until a malted cereal of enhanced enzymatic activity is formed. Generally the combination is made by inoculating the moistened cereal with the activated spores, but the activated spores and cereal may be combined before or after the moistening of the cereal.

In the process of the invention, the combination of wetted cereal and activated spores has a concentration of activated spores, holding time and holding temperature which are effective for providing the malted cereal with an increase in enzymatic activity of at least one enzyme, such as β -glucanase, xylanase, amylase, naturally occurring enzymes, and/or protease activity, which is greater than the enzymatic activity which is obtained by holding the wetted cereal without activated spores, or even with the bacteria or moulds from which the spores come.

In an important aspect, the cereal, activated spores are combined before or after the time of wetting the cereal and the combination is held at a temperature of at least about 5°C and not more than about 30°C, preferably between about 10° to about 20°C and the activated spores are at a concentration in the combination to obtain an increase in enzymatic activity of the malted cereal. In another important aspect, the wetted or moistened cereal and activated spore combination is held for a time and temperature until the cereal has a moisture content of at least about 20 weight percent. In yet another aspect, after the wetted cereal has attained an increased moisture content and has started to germinate, it is dried to a moisture content of not more than about 15 weight percent. In still another important aspect, the moistened cereal and activated spores are held together until the cereal has a moisture content of between about 20 to about 60 weight percent, preferably from about 38 to about 47 weight

Preferably, for the preparation of malted barley, the spores from bacteria are from gram positive bacteria or gram negative bacteria, selected from the group of *Micrococcus* spp., *Streptococcus* spp., *Leuconostoc* spp., *Pediococcus* spp. preferentially *Pediococcus halophilus*, *Pediococcus cerevisiae*, *Pediococcus damnosus*, *Pediococcus hemophilus*, *Pediococcus parvulus*, *Pediococcus soya*, *Lactococcus* spp., *Lactobacillus* spp. preferentially *Lactobacillus acidophilus*, *Lactobacillus amylovorus*, preferentially *Lactobacillus amylovorus* strain ATCC 33620, *Lactobacillus bavaricus*, *Lactobacillus bifementans*, *Lactobacillus brevis* var *lindneri*, *Lactobacillus casei* var *casei*, *Lactobacillus delbrueckii*, *Lactobacillus delbrueckii* var *lactis*, *Lactobacillus delbrueckii* var *bulgaricus*, *Lactobacillus fermenti*, *Lactobacillus gasserii*, *Lactobacillus helveticus*, *Lactobacillus hilgardii*, *Lactobacillus reuterii*, *Lactobacillus sake*, *Lactobacillus sativorus*, *Lactobacillus*

cremoris, *Lactobacillus kefir*, *Lactobacillus pentoceticus*, *Lactobacillus cellobiosus*, *Lactobacillus bruxellensis*, *Lactobacillus buchneri*, *Lactobacillus coryneformis*, *Lactobacillus confusus*, *Lactobacillus florentinus*, *Lactobacillus viridescens*, *Corynebacterium* spp., *Propionibacterium* spp., *Bifidobacterium* spp., *Streptomyces* spp., *Bacillus* spp., preferentially *Bacillus subtilis* strain ATCC 6051, preferentially *Bacillus circulans*, *Sporolactobacillus* spp., *Acetobacter* spp., *Agrobacterium* spp., *Alcaligenes* spp., *Pseudomonas* spp., preferentially *Pseudomonas amylophila*, *Pseudomonas aeruginosa*, *Pseudomonas cocovenenans*, *Pseudomonas mexicana*, *Pseudomonas pseudomallei*, *Gluconobacter* spp., *Enterobacter* spp., *Erwinia* spp., *Klebsiella* spp., and *Proteus* spp.

Preferably, for the preparation of malted barley spores are from fungi which are selected from the group (genera as described by Ainsworth and Bisby's dictionary of the fungi, 8th edition, 1995, edited by DL Hawkworth, PM Kirk, BC Sutton, and DN Pegler (632 pp) Cab International) of Ascomycota preferentially Dothideales, preferentially Mycosphaerellaceae preferentially *Mycosphaerella* spp., Venturiaceae preferentially *Venturia* spp., Eurotiales preferentially Monascaceae preferentially *Monascus* spp., Trichocomaceae preferentially *Emericilla* spp., *Euroteum* spp., *Eupenicillium* spp., *Neosartorya* spp., *Talaromyces* spp., Hypocreales preferentially Hypocreaceae preferentially *Hypocrea* spp., Saccharomycetales preferentially Dipodascaceae, *Dipodascus* spp., *Galactomyces* spp., Endomycetaceae preferentially *Endomyces* spp., Metschnikowiaceae preferentially *Guilliermondella* spp., Saccharomycetaceae preferentially *Debaryomyces* spp., *Dekkera* spp., *Pichia* spp., preferentially *Pichia anomala*, preferentially *Pichia anomala* strain ATCC 8168, *Kluyveromyces* spp., *Saccharomyces* spp., *Torulaspora* spp., *Zygosaccharomyces* spp., Saccharomycodaceae preferentially *Hanseniaspora* spp.; Schizosaccharomycetales preferentially Schizosaccharomycetaceae preferentially *Schizosaccharomyces* spp., Sordariales preferentially

Chaetomiaceae, Chaetomium spp., preferentially Chaetomium virens strain ATCC 32319, Sordariaceae preferentially Neurospora spp., Zygomycota preferentially Mucorales preferentially Mucoraceae preferentially Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Chlamydomucor spp., Mucor spp. preferentially Mucor circinellus, Mucor grisecyanus, Mucor hiemalis, Mucor indicus, Mucor mucedo, Mucor piriformis, Mucor plumbeus, Mucor praini, Mucor pusillus, Mucor silvaticus, Mucor javanicus, Mucor racemosus, Mucor rouxianus, Mucor rouxii, Mucor aromaticus, Mucor flavus, Mucor miehei, Rhizopus spp. preferentially Rhizopus arrhizus, Rhizopus oligosporus, Rhizopus oryzae preferentially Rhizopus oryzae strain ATCC 4858, Rhizopus oryzae strain ATCC 9363, Rhizopus oryzae strain NRRL 1891, Rhizopus oryzae strain NRRL 1472, Rhizopus stolonifer, Rhizopus thailandensis, preferentially Rhizopus thailandensis strain ATCC 20344, Rhizopus formosensis, preferentially Rhizopus formosensis strain ATCC 26612, Rhizopus chinensis, Rhizopus cohnii, Rhizopus japonicus, Rhizopus nodosus, Rhizopus delemar, Rhizopus acetorinus, Rhizopus chlamydosporus, Rhizopus circinans, Rhizopus javanicus, Rhizopus peka, Rhizopus salto, Rhizopus tritici, Rhizopus niveus, Rhizopus microsporus, Mitosporic fungi preferentially Aureobasidium spp., Acremonium spp., Cercospora spp., Epicoccum spp., Monilia spp. preferentially Monilia candida, Monilia sitophila, Mycodermia spp., Candida spp., preferentially Candida diddensiae, Candida edax, Candida etchellsii, Candida kefir, Candida krisel, Candida lactosa, Candida lambica, Candida melinii, Candida utilis, Candida milleri, Candida mycoderma, Candida parapsilosis, Candida obtux, Candida tropicalis, Candida valida, Candida versatilis, Candida guilliermondii, Rhodotorula spp., Torulopsis spp., Geotrichum spp. preferentially Geotrichum amycelium, Geotrichum amillarum, Geotrichum asteroides, Geotrichum bipunctatum, Geotrichum dulcitum, Geotrichum ericense, Geotrichum fici, Geotrichum flavo-brunneum, Geotrichum fragrans, Geotrichum gracile, Geotrichum heritum, Geotrichum klebakii, Geotrichum penicillatum,

Geotrichum hirtum, *Geotrichum pseudocandidum*, *Geotrichum rectangulatum*, *Geotrichum suaveolens*, *Geotrichum vandyiae*, *Geotrichum loubieri*, *Geotrichum microsporum*, *Cladosporium* spp., *Trichoderma* spp. preferentially *Trichoderma hamatum*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma pseudokoningii*, *Trichoderma reesei*, preferentially *Trichoderma reesei* strain ATCC 5675, *Trichoderma virgatum*, *Trichoderma viride*, *Oldium* spp., *Alternaria* spp. preferentially *Alternaria alternata*, *Alternaria tenuis*, *Helminthosporium* spp. preferentially *Helminthosporium gramineum*, *Helminthosporium sativum*, *Helminthosporium teres*, *Aspergillus* spp. preferentially *Aspergillus ochraceus* Group, *Aspergillus nidulans* Group, *Aspergillus versicolor* Group, *Aspergillus wentii* Group, *Aspergillus candidus* Group, *Aspergillus flavus* Group, *Aspergillus niger* Group, *Aspergillus oryzae* strain ATCC 14156, *Penicillium* spp. preferentially *Penicillium aculeatum*, *Penicillium citrinum*, *Penicillium claviforme*, *Penicillium funiculosum*, *Penicillium italicum*, *Penicillium lanoso-viride*, *Penicillium emersonii*, *Penicillium lilacinum*, *Penicillium expansum*. and mixtures thereof.

Preferably, for the preparation of malted cereals other than malted barley, especially for the preparation of malted wheat, rye, corn, oats, rice, millet, triticale, and sorghum, said bacteria are gram positive or gram negative bacteria selected from the group of *Micrococcus* spp., *Streptococcus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Lactococcus* spp., *Lactobacillus* spp., *Corynebacterium* spp., *Propionibacterium* spp., *Bifidobacterium* spp., *Streptomyces* spp., *Bacillus* spp., *Sporolactobacillus* spp., *Acetobacter* spp., *Agrobacterium* spp., *Alcaligenes* spp., *Pseudomonas* spp., *Gluconobacter* spp., *Enterobacter* spp., *Erwinia* spp., *Klebsiella* spp., *Proteus* spp. or a mixture thereof; and said fungi are fungi selected from the group of: Ascomycota preferentially Dothideales preferentially Mycophaerellaceae preferentially *Mycosphaerella* spp., *Venturiaceae* preferentially *Venturia* spp.; Eurotiales preferentially Monascaceae preferentially *Monascus* spp., Trichocomaceae preferentially *Emericella* spp., *Eurotium* spp., *Eupenicillium* spp., *Neosartorya* spp., *Talaromyces* spp., *Hypocreales*

preferentially Hypocreaceae preferentially Hypocrea spp., Saccharomycetales preferentially Dipodascaceae preferentially Dipodascus spp., Galactomyces spp., Endomycetaceae preferentially Endomyces spp., Metschnikowiaceae preferentially Guilliermondella spp., Saccharomycetaceae preferentially Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccaromycodaceae preferentially Hanseniaspora spp., Schizosaccharomycetales preferentially Schizosaccharomycetaceae preferentially Schizosaccharomyces spp.; Sordariales preferentially Chaetomiaceae preferentially Chaetomium spp., Sordariaceae preferentially Neurospora spp., Zygomycota preferentially Mucorales preferentially Mucoraceae preferentially Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Clamydomucor spp., Mucor spp., Rhizopus spp.; preferentially Rhizopus oryza strain ATCC 9363, Mitosporic fungi preferentially Aureobasidium spp., Acremonium spp., Cerocospora spp., Epicoccum spp., Monilia spp., Mycoderma spp., Candida spp., Rhodotorula spp., Torulopsis spp., Geotrichum spp., Cladosporium spp., Trichoderma spp., Oldium spp., Alternaria spp., Helminthosporium spp., Aspergillus spp., Penicillium spp.

DEFINITIONS

As used herein the term "spore" refers to a dormant and highly resistant reproductive cell formed by bacteria and fungi in response to environment conditions that do not favor the growth of the organism. When exposed to favorable environmental conditions, spores are capable of developing into a viable adult organism without fusion with another cell.

As used herein the term "activated spore" means a spore having one of the following properties:

i) The spore is swollen such that its size is increase by a factor of between about 1.2 and about 10 over its dormant size; and/or

ii) one or more germ tubes per spore is formed.

Activated spores are prepared by one or a combination of the following treatments.

i) cycles of wetting and/or drying;

ii) addition of appropriate nutritional supplies (such as a nitrogen source, preferably amino acids and/or a carbon source, preferably mono- or disaccharides) or spore elements;

iii) exposure to temperature changes, preferably within a temperature range of about 0° to about 80°C.

iv) exposure to changes in pH, preferably within a pH range of about 2.0 to about 8.0, more preferably about 3.0 to about 6.0.

The term "germination" as used herein means the beginning or resumption of growth by a seed. In accordance with the process of the present invention, germination begins to occur during and/or after the cereal has been steeped. Germination of cereals is generally understood to mean hydration of the seed, swelling of the cereal and inducing growth of the embryo. Environmental factors affecting germination include moisture, temperature and oxygen level. A rapid increase in cells of the root stem leads to root development, while corresponding growth sends forth a shoot.

As used herein, the term "steeping" refers to wetting of the cereal. Wetting may include one or more stages over a time and temperature effective for providing a moisture content of between about 20% and about 60% by weight.

The term "specific activity" as used herein refers to the concentration and activity of an enzyme in a preparation. The specific activity of a preparation is reported as units/mg protein. One unit of enzyme is that amount that catalyzes the formation of 1 mmole of product per minute under defined conditions. The

amount of enzyme present in a preparation is measured using standard protein assay techniques and catalytic activity is determined by following the formation of product or removal of substrate over time.

Description of the Preferred Embodiments

According to a preferred embodiment, the preparation process of malted cereals according to the invention comprises the following steps: a steeping step includes one or more wetting stages or the total time of submersion in water during steeping for physiological reasons does not exceed 30 hours (preferably 10 to 25 hours) or the kilning step includes more than two temperature steps and the microbial cultures which are added, are preferably selected from the group consisting of *Rhizopus* spp., preferably *Rhizopus oryzae*, such as *Rhizopus oryzae* strain ATCC9363 and/or *Pseudomonas* spp., preferably *Pseudomonas herbicola*.

According to the invention, the malted cereals are selected from the group of barley, wheat, rye, corn, oats, rice, millet, triticale, sorghum and the like.

In the process according to the invention, the same or different activated spores are added in one or more time(s). The use of activated spores greatly enhances their contribution to improved malt quality, most likely because of more vigorous growth. The activated spores have one of the following properties: the treated spores are significantly more swollen than their dormant size, more particularly, the size of the spores is increased by a factor preferably between 1.2 and 10 over their dormant size and/or one or more germ tubes per spore are formed. The activated spores are prepared by subjecting them to environmental changes, preferably by one or a combination of the following treatments:

- (a) cycles of wetting and/or drying;
- (b) addition of appropriate nutritional supplies (such as a nitrogen source, preferably amino acids and/or a carbon source, preferably mono- or disaccharides) or spore elements;

- (c) exposure to temperature changes, preferably within a temperature range of 0° to 80°C;
- (d) exposure to changes in pH, preferably within a pH range of 2.0 to 8.0, more preferably between 3.0 and 6.0.

The activated spores may be introduced before or during the malting process. For example, the activated spores may be introduced during the various malting or steeping stages before or after immersion of the cereal.

The concentration of the spores may vary depending on the conditions of the malting process and the type of active spore being utilized. Generally about 1×10^2 to about 1×10^7 , preferably about 1×10^3 to about 1×10^6 activated spores per gram air dry cereal is utilized.

The present invention also concerns the malted cereals obtained according to the process of the invention, which present improved European Brewery Convention analysis results. Said improvements may have to do with modification and/or increased hydrolytic enzyme activities. At the same time, a decreased level of toxins, an increased microbial safety by e.g., outcompeting undesirable microbial flora such as *Fusarium* and/or an increased acceptability compared to the malted cereals according to the state of the art, may be observed.

For instance, the malted cereals according to the invention may have a lower β -glucan content or a higher enzyme activity such as, for example, β -glucanase or xylanase activity (as represented in the following examples and figures) than the malted cereals according to the state of the art. This allows for a better processability of the malt in wort and beer production as exemplified by increased rates of filtration.

The activated spores and cereal may be combined and wetted by submersion in water to steep the combination which should not exceed 30 hours. The activated spores can also be sprayed on the barley during the steep period or during the germination process. The pH during this period should be from

about 1.5 to about 14, preferably from about 4 to about 6. β -glucanase activity of malted barley made according to the invention is higher than 700 units/kg and xylanase activity is higher than 250 units/kg.

An object of the present invention concerns the use of the malted cereals according to the invention for the preparation of beverages.

The invention is also related to these improved beverages.

The improved malted cereals according to the invention could also be used in other biotechnological processes well known by the Person Skilled in the Art, in which in most cases advantage is taken of their improved quality.

The present invention will be further described in various examples in view of the following drawings.

Brief Description of the Drawings

- Figure 1 represents the β -glucanase activity of malted barley obtained according to the preparation process of example 1. (legend: see example 1).
- Figure 2 represents the xylanase activity of malted barley obtained according to the preparation process of example 1. (legend: see example 1).
- Figure 3 represents the β -glucanase activity of malted barley obtained according to the preparation process of example 3. (legend: see example 3).
- Figure 4 represents the xylanase activity of malted barley obtained according to the preparation process of example 3. (legend: see example 3).

Figure 5 represents the relative increase factor (R.I.F.) for bacterial populations (see text, malt evaluation, example 2) (legend: see example 2).

EXAMPLE 1.

1. Preparation of Microbial Cultures

Strain

- S46: *Rhizopus oryzae* ATCC 9363.

Preparation of the Spore Suspension

- the strain was grown on PDA (Potato Dextrose Agar, Oxoid) for approximately 10 days at 28°C;
- the spores were harvested by flooding the cultures with sterile physiological saline (0.9% NaCl) and by rubbing the sporulated mycelium gently with a sterile spatula;
- the spore suspension was washed twice with sterile physiological saline (0.9% NaCl) by centrifugation (5500 rpm, Sorvall type SS-34®, for 15 min.) and resuspended in sterile physiological saline (0.9% NaCl);
- the spore density was determined microscopically using a Thoma counting chamber.

Activation of the Spore Suspension

- 10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid), pH = 4.0 and incubated in a shaking water bath during 5 to 6 hours at $\pm 42^\circ\text{C}$;
- The activated spores were harvested by centrifugation (3500 rpm, Sorvall type SS-34®, for 15 min.), washed once with sterile physiological saline

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(0.9% NaCl) by centrifugation (3500 rpm, Sorvall type SS-34®, for 15 min.) and resuspended in sterile physiological saline (0.9% NaCl).

2. Barley

- Plaisant - 1994 French harvest.

3. Process

Setup

Malts were made by four different malting processes:

- *A1. traditional malting*
(without inoculation of any spore suspension)
- *B1. malting process using non-activated spores*
(Inoculation of the steeped barley with a suspension of non-activated spores of *Rhizopus oryzae* ATCC 9363)
- *C1. malting process according to the invention*
(inoculation of the steeped barley with a suspension of activated spores of *Rhizopus oryzae* ATCC 9363)
- *D1. malting process according to the invention*
(Inoculation of the steeped barley during the first wet stage with a suspension of activated spores of *Rhizopus oryzae* ATCC 9363)

Steeping

- the steeping was carried out on a 2 kg base with a total water (tap water) to air dry barley ratio of 1.5:1;
- use was made of 2 fermentors (Bioflo III, New Brunswick Scientific), in which perforated plates were placed;
- temperature was only controlled during the wet stages, during the air rest stages, the system was allowed to reach room temperature ($\pm 20^{\circ}\text{C}$);

- during the whole steeping period, the barley was aerated (4 liter sterile air per minute);
- steeping was carried out by immersion using the following scheme:

	Temperature (°C)	Duration (h)
First wet stage	13	6:00
First air rest stage	20	17:00
Second wet stage	14	5:00
Second air rest stage	20	15:30
Third wet stage	16	2:30

Addition of the Microbial Cultures

- \pm 460 g of steeped barley was immersed in 0.5 litre of tap water which contained no spores (A1), non-activated spores of *Rhizopus oryzae* ATCC 9363 (B1) or activated spores of *Rhizopus oryzae* ATCC 9363 (C1, according to the invention); for B1 and C1, the steeped barley was inoculated with 10^4 spores per gram of air dry barley;
- during the steeping, 10^4 activated spores per gram air dry barley were inoculated to the water of the first wet stage (D1);
- the fluid was removed by draining.

Germination

- germination was carried out in a cylindrical container with perforated lids at a temperature of 16°-18°C during 4 days;
- air was supplied by natural diffusion;

- the containers were slowly rotated on an electronically controlled roller system (Cellroll®, Tecnorama); i.e., every two hours the containers were rolled for 15 min. at 1 rpm.

Kilning

- the kilning was carried out in a Joe White malting unit (Australia).

	Air flow (%)	Recirc. Air (%)	Temp. (°C)	Durat. (h)
First kilning stage	25	0	62	3:00
Second kilning stage	25	0	65	2:00
Third kilning stage	25	0	68	2:00
Fourth kilning stage	25	25	73	2:00
Fifth kilning stage	25	50	78	1:00
Sixth kilning stage	25	75	80	2:00
Seventh kilning stage	25	100	83	6:00
Shut down air off				Time out

4. Methods of Analysis and Results

Methods for determination and units of moisture, extract, extract difference, color, total protein content, soluble protein content, Kolbach Index, pH, diastatic power, according to Analytica-European Brewery Convention (Fourth Edition, 1987, Brauerei und Getränke-Rundschau).

Methods for determination and units of turbidity, friability, homogeneity, whole grains, b-glucan content, according to Analytica-European Brewery

Convention (Fourth Edition, 1987, Brauerei und Getränke-Rundschau, supplement published in 1989).

Postcoloration of the wort is determined after boiling the congress wort under reflux at 108°C during 2 hours.

The viscosity of the congress wort is determined with the Delta-viscosimeter.

For the determination of the filtration volume, the congress wort is filtered over a Schleicher and Schuell 597 1/2 folded filter. The volume (in ml) that is obtained after 1 hour of filtration is the filtration volume of the wort.

Modification is determined with the Calcofluor apparatus (Haffmans) according to the Carlsberg method (Analytica-European Brewery Convention (Fourth Edition, 1987, Brauerei und Getränke-Rundschau).

The β -glucanase and xylanase activities are determined with the β -glucazym method (Megazyme (Austr.) Pty Ltd. (April, 1993) and the xylazym method ((Megazyme (Austr.) Pty Ltd. (September 1995)), respectively.

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	Traditional Malting Process (A1)	Malting Process using non- activated spores (B1)	Malting Process according to the Invention (C1)	Malting Process according to the Invention (D1)
Moisture	3.9	4.1	3.8	4.3
Extract	80.3	80.4	80.3	79.8
Extract Difference	0.8	0.8	0.4	1.1
Color	3.3	3.3	4.1	4.1
Wort Turbidity	1.3	1.2	0.7	0.8
Postcoloration	6	6	7.3	7.5
Total Protein Content	10.1	10.3	10	10.1
Soluble Protein Content	4.1	4.4	4.8	5.2
Kolbach Index	40.6	42.7	48	51.0
Viscosity	1.57	1.52	1.52	1.54
pH	6.05	6.3	5.87	6.79
Diastatic Power	345	349	352	419
Whole Grains	0.3	0.3	0.1	ND
Friability	83	82	83.9	ND
Homogeneity	98.5	97.9	98.6	ND
β -glucan content	122	108	46	<40
Filtration Volume	210	265	290	275
Modification	88.2	90.5	93.4	ND
β -glucanase Activity	214	371	683	3856
Xylanase Activity	28	34	56	984

Figures 1 and 2 represent the β -glucanase and xylanase activity, respectively of the obtained malted barley (A1, B1, C1, D1). These malted barleys are obtained according to a traditional malting process (A1) or using non-activated spores during the malting process (B1) or according to the above-described malting process of the invention (C1, D1). The β -glucanase activity was determined with the β -glucazym method (Megazyme (Austr.) Pty Ltd. (April, 1993)). Therefore, malt β -glucanase activity (U/kg) was calculated at $380 \times E(590 \text{ nm}) + 20$. The xylanase activity was determined with the endo 1-4-xylazym method (Megazyme (Austr.) Pty Ltd. (September 1995)). Therefore, malt xylanase activity (U/kg) was calculated as $(46.8 \times E(590 \text{ nm}) + 0.9) \times 5$.

EXAMPLE 2.

1. Preparation of Microbial Cultures

Strain

- S46: *Rhizopus oryzae* ATCC 9363.

Preparation of the Spore Suspension

- as described in Example 1.

Activation of the Spore Suspension

- as described in Example 1.

2. Barley

- Stander - 1995 North American harvest.

3. Process

Setup

Malts were made by six different malting processes:

- **A2. traditional malting process**
(without inoculation of any spore suspension)
- **B2. malting process using non-activated spores**
(inoculation of the steeped barley with a suspension of non-activated spores of *Rhizopus oryzae* ATCC 9363)
- **C2. malting process according to the invention**
(inoculation of the steeped barley during the first wet stage with a suspension of activated spores of *Rhizopus oryzae* ATCC 9363)
- **D2. malting process according to the invention**
(inoculation of the steeped barley during the second wet stage with a suspension of activated spores of *Rhizopus oryzae* ATCC 9363)
- **E2. malting process according to the invention**
(inoculation of the steeped barley during the third wet stage with a suspension of activated spores of *Rhizopus oryzae* ATCC 9363)
- **F2. malting process according to the invention**
(inoculation of the steeped barley with a suspension of activated spores of *Rhizopus oryzae* ATCC 9363)

Steeping and Addition of the Microbial Cultures

- the steeping was carried out on a 300 g base with a total water (tap water) to air dry barley ratio of 5:3;
- use was made of 2000 ml flasks;
- a temperature of 18°C was maintained during the wet stages and during the air rest stages;
- during the whole steeping period, the barley was aerated by means of compressed air;
- steeping was carried out by immersion using the following schedule:

	Duration (h)
First wet stage	6:00
First air rest stage	18:00
Second wet stage	5:00
Second air rest stage	19:00
Third wet stage	2:00

- during the steeping, 10^4 activated spores per gram of air dry barley were inoculated to the water of the first wet stage (C2), of the second wet stage (D2) or of the third wet stage (E2) before immersion of the barley;
- the steeped barley was immersed in 0.5 liters of tap water which contained no spores (A2), non-activated (B2) or activated (C2, D2, E2, F2) spores;
- for B2, and F2, the steeped barley was inoculated with 10^4 spores per gram of air dry barley,
- the fluid was removed by draining.

Germination

- as described in Example 1

Kilning

- as described in Example 1

Malt evaluation

Determination of the Increase of the Bacterial Population

To judge the evolution of the bacterial population during the malting process, a relative increase factor (R.I.F.) was determined by dividing the total

bacterial count occurring on the green malt by the total bacterial count occurring on the barley. The total bacterial count was determined after plating appropriate dilutions of an extract of the kernels on Tryptic Soy Agar (Oxoid) supplemented with 100 ppm pimarcine and after incubation at 28° C for 3 days.

Figure 5 shows the increase of the bacterial population during the malting according to the preparation process of Example 2.

EXAMPLE 3

1. Preparation of Microbial Cultures

Strain

- S 46: *Rhizopus oryzae* ATCC 9363

Preparation of the Spore Suspension

- as described in Example 1

Activation of the Spore Suspension

- as described in Example 1

2. Barley

- Plaisant - 1994 French harvest;

3. Process

Setup

Malts were made by three different malting processes:

- A3. *traditional malting*
(without inoculation of any spore suspension)

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- **B3. malting process using non-activated spores**
(inoculation of the steeped barley with a suspension of non-activated spores of *Rhizopus oryzae* ATCC 9363)
- **C3. malting process according to the invention**
(inoculation of the steeped barley with a suspension of activated spores of *Rhizopus oryzae* ATCC 9363)

Steeping

- the steeping was carried out on a 2 kg base air dry barley with a total water (tap water) to air dry barley ratio of 1.5:1;
- the pH of the steeping water was controlled at pH = 5.5 by addition of lactic acid and NaOH;
- a fermentor (Bioflo III, New Brunswick Scientific), in which a perforated plate was placed, was used for steeping;
- temperature was only controlled during the wet stages; during the air rest stages the system was allowed to reach room temperature (ca. 20° C);
- during the whole steeping period the barley was aerated (4 liters sterile air per minute);
- steeping was carried out by immersion using the following schedule:

	Temperature (° C)	Duration (h)
First wet stage	13	6:00
First air rest stage	20	17:00
Second wet stage	14	5:00
Second air rest stage	20	15:30
Third wet stage	16	2:30

Addition of the Microbial Cultures

- 460 g of steeped barley was immersed in 0.5 liters of tap water which contained no spores (A3), non-activated spores of *Rhizopus oryzae* ATCC 9363 (B3) or activated spores of *Rhizopus oryzae* ATCC 9363 (C3 according to the invention); for B3 and C3, the steeped barley was inoculated with 10^4 spores per gram of air dry barley;
- the fluid was removed by draining.

Germination

- as described in Example 1

Kilning

- as described in Example 1

4. Methods of analysis and results

These were as described in Example 1 (4. Methods of Analysis and Results).

See table on next page. In this table:

- A1/3: Traditional malting process
- B1/3: Malting process using non-activated spores
- C1/3: Malting process according to the invention

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	Example 3					Example 1			
	pH control of the steeping water (pH - 5.5)					No pH control of the steeping water			
	A3	B3	C3	A1	B1	C1			
Moisture	3.8	3.8	3.7	3.9	4.1	3.8			
Extract	78.9	80.2	80.7	80.3	80.4	80.3			
Extract Difference	0.6	0.7	0.4	0.8	0.8	0.4			
Color	3.2	4.2	4.4	3.3	3.3	4.1			
Wort Turbidity	1	1	0.8	1.3	1.2	0.7			
Postcoloration	5.1	7	7.2	6	6	7.3			
Total Protein Content	10.2	10.1	10	10.1	10.3	10			
Soluble Protein Content	4	4.4	4.8	4.1	4.4	4.8			
Kolbach Index	39.2	43.6	48	40.6	42.7	48			
Viscosity	1.52	1.53	1.52	1.57	1.52	1.52			
pH	6.02	5.97	5.91	6.05	6.03	5.87			
Diastatic Power	348	333	355	345	349	352			
Whole Grains	0.2	0.2	0.1	0.3	0.3	0.1			
Friability	81	81	85	83	82	83.9			
Homogeneity	97.6	97.8	98.9	98.5	97.9	98.6			
β-glucan content	190	57	40	122	108	46			
Filtration Volume	210	215	200	210	265	290			
Modification	84.1	85.5	87.4	88.2	90.5	93.4			
β-glucanase Activity	202	931	1322	214	371	683			
Xylanase Activity	43	65	71	28	34	56			

Figure 3 represents the β -glucanase activity, measured according to β -Glucazym method [Megazyme (AUSTR) Pty. Ltd.] of the malted cereals A3, B3 and C3. Malt β -glucanase activity (U/kg) was calculated as described in example 1. A3 was obtained by the traditional malting process with pH control of the steeping water (pH = 5.5). B3 resulted from the malting process according to the invention with the inoculation of steeped barley with a suspension of non-activated spores of *Rhizopus oryzae* ATCC 9363 and with pH control of the steeping water (pH = 5.5). C3 was obtained by the malting process according to the invention with the inoculation of the steeped barley with a suspension of activated spores of *Rhizopus oryzae* ATCC 9363 and with pH control of the steeping water (pH = 5.5).

These results show the increased β -glucanase activity when the pH of the steeping water is maintained at around 5.5.

Figure 4 gives the corresponding results for xylanase activity. These were measured according to xylazym method, Megazyme ((AUSTR) Pty. Ltd. (September 1995)). Malt xylanase activity was calculated as described in Example 1.

Comparison of the β -glucanase activity obtained according to examples 1 and 3 with the β -glucanase activity according to the state of the art as described in WO94/29430

In order to compare the improved results regarding β -glucanase activity by the present invention, we defined the factor m as follows:

$$m = \frac{\beta\text{-glucanase activity of the treated malt}}{\beta\text{-glucanase activity of the controlled malt}}$$

This factor was calculated for control malt and malted treated with *Rhizopus oryzae* ATCC 9363 as described in Examples 1 and 3 of the present invention.

It was also calculated for the data described in WO94/29430 (Example 1) where *Geotrichum candidum* was used.

Both as described in WO94/29430, and in the present application, β -glucanase activity was determined with the beta-glucazyme method [Megazyme (AUSTR) Pty. Ltd. (April 1993)]. Therefore, malt β -glucanase activity (U/kg) was calculated as $380 \times E(590nm) + 20$ and one unit of activity was defined as the amount of enzyme required to release one micromole of reducing sugar equivalents per minute under the defined above conditions.

Comparison of the Results:

State of the Art				Invention			
	m		m	Ex. 1	m	Ex. 3	m
Gc *	1.48	Gc *	1.98	B1/A1	1.73	B3/A3	4.61
				C1/A1	3.19	C3/A3	6.54
				D1/A1	18.02		

*Gc: *Geotrichum candidum*

The results clearly show that the present invention provides for a more drastic increase in malt β -glucanase activity than that described earlier (WO 94/29430).

It thus appears that it is possible to obtain malted cereals having a β -glucanase activity increased by at least a factor 4 compared to the conventional malting process wherein the addition of microbial culture is omitted.

From Figure 2 and 4, it also appears that it is possible to obtain malted cereals having a xylanase activity increased by at least a factor 4 compared to conventional malting process wherein the addition of microbial culture is omitted.

EXAMPLE 4

1. Preparation of the microbial cultures

Strain

- S40: *Aspergillus oryzae* ATCC 14156

Preparation of the spore suspension

- the strain was grown on PDA (Potato Dextrose Agar, Oxoid) for approximately 7 days at 28° C;
- the spores were harvested by flooding the culture with sterile physiological saline (0.9% NaCl) and by rubbing the sporulated mycelium gently with a sterile spatula;
- the spore suspension was washed once with sterile physiological saline (0.9% NaCl) by centrifugation (5500 rpm, Sorval type SS-34@, for 15 min) and resuspended in sterile physiological saline (0.9% NaCl);
- the spore density was determined microscopically using a Thoma counting chamber.

Activation of the Spore Suspension

- 5×10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid), pH=5.0 and incubated in a shaking water bath during 3 hours (1) or 1 hour (2) at 35° C,

2. Cereal

- Clarine barley - 1995 French harvest

3. Process

Malts were made by two different malting processes:

- A4. traditional malting
(without inoculation of any spore suspension)
- E4. malting process according to the invention
(inoculation of the steeped barley during the first and third wet stage with a suspension of activated spores of *Aspergillus oryzae* ATCC 14156)

Steeping

as described in Example 1

Addition of the microbial cultures

- during the steeping, 5×10^3 activated spores (1) per gram air dry barley were inoculated to the water of the first wet stage and 10^4 activated spores (2) per gram air dry barley were inoculated to the water of the third wet stage (E4);

Germination

- germination of ± 460 g steeped barley was carried out in cylindrical containers with perforated lids at a temperature of 16° - 18° C during 4 days;
- air was supplied by natural diffusion;
- the containers were slowly rotated on an electronically controlled roller system (Cellroll®, Tecnorama); i.e., every two hours the containers were rolled for 15 min at 1 rpm.

Kilning

as described in Example 1

4. Methods of analysis and results

These were described in Example 1 (4, Methods of Analysis and Results) Method for the determination of the acrospire length according to Analytica-European Brewery Convention (Fourth Edition, 1987, Brauerei und Getränke-Rundschau).

		0	0-½	½-¾	¾-1	>1
1 day germination	A4	0	1	60	39	0
1 day germination	E4	0	0	11	77	12
4 days germination	A4	1	1	31	64	3
4 days germination	E4	1	0	1	42	49

	Traditional Malting Process (A4)	Malting Process According to the Invention (E4)
Moisture	4.3	4.0
Extract	80.8	81.1
Extract Difference	1.0	0.3
Color	2.8	3.2
Wort Turbidity	1.6	1.0
Postcoloration	4.8	5.4
Total Protein Content	10.1	10.0
Soluble Protein Content	3.9	4.5
Kolbach Index	38.6	44.7
Viscosity	1.57	1.48
pH	5.98	5.89
Diastatic Power	197	201
Whole Grains	1.3	0.6
Friability	81	89
Homogeneity	95.0	98.4
β -glucan Content	378	132
Filtration Volume	300	310
Modification	83.9	89.8
β -glucanase Activity	309	392
Xylanase Activity	27.82	17.52

EXAMPLE 5

1. Preparation of the Microbial Cultures

Strains

- S40: *Aspergillus oryzae* ATCC 14156
- S46: *Rhizopus oryzae* ATCC 9363

Preparation of the Spore Suspensions

As described in Example 4

Activation of the Spore Suspensions

S40:

- 5 x10⁷ spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid) pH=5.0 and incubated in a shaking water bath during 1 hour at 35°C;
- the activated spores were harvested by centrifugation (3500 rpm, Sorvall type SS-34®, for 15 min.) and resuspended in sterile physiological saline (0.9% NaCl).

S45

- 5 x10⁷ spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid) pH=4.0 and incubated in a shaking water bath during 5 hours at 42°C;
- the activated spores were harvested by centrifugation (3500 rpm, Sorvall type SS-34®, for 15 min.) and resuspended in sterile physiological saline (0.9% NaCl).

2. Cereal

- Clarine - 1995 French harvest

3. Process

Setup

Malts were made by two different malting processes:

- A5. *traditional malting*
(without inoculation of any spore suspension)

- **F5. malting process according to the invention**
(inoculation of the steeped barley during the first wet stage with a suspension of activated spores of *Aspergillus oryzae* ATCC 14156 and after steeping with a suspension of activated spores of *Rhizopus oryzae* ATCC 9363)

Steeping

As described in Example 1

Addition of the Microbial Cultures

- during steeping, 10^4 activated spores of *Aspergillus oryzae* ATCC 14156 per gram air dry barley were inoculated to the water of the first wet stage (F5, according to the invention);
- \pm 460 g of steeped barley was immersed in 0.5 liters of tap water which contained no spores (A5) or activated spores of *Rhizopus oryzae* ATCC 9363 (F5, according to the invention); for F5 the steeped barley was inoculated with 10^4 activated spores per gram air dry barley;
- the fluid was removed by draining.

Germination

As described in Example 4.

Kilning

As described in Example 1.

4. Methods of Analysis and Results

These were as described in Example 1 (4. Methods of Analysis and Results).

Method for the determination of the acrospire length according to Analytica-European Brewery Convention (Fourth Edition, 1987, Brauerei und Getränke-Rundschau).

		0	0-¼	¼-½	½-¾	¾-1	>1
1 day germination	A5	1	1	53	44	1	0
1 day germination	F5	0	1	21	73	5	0
4 days germination	A5	0	0	0	29	63	8
4 days germination	F5	0	0	0	13	63	24

It was noted that the use of activated spores of *Aspergillus oryzae* ATCC improved the malt analytical specifications.

Furthermore, it was found that during the malting process, the barley acrospire lengths were significantly longer using the process according to the invention in comparison to the traditional malting process.

	Traditional Malting Process (A5)	Malting Process According to the Invention (F5)
Moisture	3.9	4.2
Extract	81.4	81.8
Extract Difference	0.9	1.1
Color	3.8	3.8
Wort Turbidity	1.4	1.0
Postcoloration	6.9	6.4
Total Protein Content	10.1	10.2
Soluble Protein Content	4.8	5.2
Kolbach Index	48.0	51.3
Viscosity	1.51	1.50
pH	5.88	5.82

(continuing)	Traditional Malting Process (A5)	Malting Process According to the Invention (F5)
Diastatic Power	199	214
Whole Grains	0.8	1.1
Friability	89	95
Homogeneity	98.3	98.3
β -glucan Content	120	51
Filtration Volume	270	220
Modification	96.8	98.6
β -glucanase Activity	263	907
Xylanase Activity	28.86	57.76

EXAMPLE 6

1. Preparation of the Microbial Cultures

Strains

- S46: *Rhizopus oryzae* ATCC 9383

Preparation of the Spore Suspensions

As described in Example 4

Activation of the Spore Suspensions

- 5×10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid) pH=4.0 and incubated in a shaking water bath during 5 hours at 42°C;

- the activated spores were harvested by centrifugation (3500 rpm, Sorvall type SS-340, for 15 min.) and resuspended in sterile physiological saline (0.9% NaCl).

2. Cereal

- Wheat: Mobil - 1996 Belgian harvest

3. Process

Setup

Malts were made by two different malting processes:

- A6. *traditional malting*
(without inoculation of any spore suspension)
- D6. *malting process according to the invention*
(inoculation of the steeped wheat during the first wet stage with a suspension of activated spores of *Rhizopus oryzae* ATCC 9363)

Steeping

- the steeping was carried out in a 2 kg base with a total water (tap water) to air ratio of 1.5:1;
- use was made of 2 fermentors (Bioflo III, New Brunswick Scientific), in which a perforated plate was placed;
- temperature was only controlled during the wet stages; during the air rest stages, the system was allowed to reach room temperature ($\pm 20^{\circ}\text{C}$);
- during the whole steeping period the wheat was aerated (4 liter sterile air per minute);
- steeping was carried out by immersion using the following scheme:

	Temperature (°C)	Duration (h)
First wet stage	13	6:00
First air rest stage	20	16:00
Second wet stage	14	4:00
Second air rest stage	20	16:00
Third wet stage	16	2:00

Addition of the Microbial Cultures

- during steeping, 10^4 activated spores of per gram air dry wheat were inoculated to the water of the first wet stage (D6);

Germination

As described in Example 4.

Kilning

As described in Example 1.

4. Methods of Analysis and Results

These were as described in Example 1 (4. Methods of Analysis and Results).

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	Traditional Malting Process (A6)	Malting Process According to the Invention (D6)
Moisture	5.5	5.4
Extract	83.6	85.5
Extract Difference	1.0	0.6
Color	3.9	7.6
Wort Turbidity	1.4	1.4
Postcoloration	5.8	11.5
Total Protein Content	14.0	14.8
Soluble Protein Content	4.9	9.7
Kolbach Index	35.0	65.5
Viscosity	1.99	1.79
pH	6.02	5.63
Diastatic Power	183	193
Whole Grains	19.4	20.2
Friability	35	42
Homogeneity	79.4	78.7
Filtration Volume	220	295
β -glucanase Activity	10.9	16,640
Xylanase Activity	16.85	1,620.1

EXAMPLE 7

1. Preparation of the Microbial Cultures

Strains

- S46: *Rhizopus oryzae* ATCC 9363

Preparation of the Spore Suspension

- the strain was grown on PDA (Potato Dextrose Agar, Oxoid) for approximately 7 days at 28°C;
- the spores were harvested by flooding the culture with sterile physiological saline (0.9% NaCl) and by rubbing the sporulated mycelium gently with a sterile spatula;
- the spore suspension was washed once with sterile physiological saline (0.9% NaCl) by centrifugation (3500 rpm, Jouan C312, for 15 min.) and resuspended in sterile physiological saline (0.9% NaCl);
- the spore density was determined microscopically using a Thoma counting chamber.

Activation of the Spore Suspension

- 5×10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid) pH=4.0 and incubated in a shaking water bath during 5 hours at 42°C.

2. Cereal

- Sorghum (S14)

3. Process

Setup

Malts were made by two different malting processes:

- A7. *traditional malting*
(without inoculation of any spore suspension)
- D7. *malting process according to the invention*
(inoculation of the sorghum during the first wet stage with a suspension of activated spores of *Rhizopus oryzae* ATCC 9363).

Cleaning

- washing of the sorghum is performed by using 6 liters tap water per kilogram sorghum and by removing the excess water.

Steeping

- the steeping was carried out in a 2 kg base with a total water (tap water) to air ratio of 1.5:1;
- use was made of 2 fermentors (Bioflo III, New Brunswick Scientific), in which a perforated plate was placed;
- temperature was only controlled during the wet stages; during the air rest stages, the system was allowed to reach room temperature ($\pm 20^{\circ}\text{C}$);
- during the whole steeping period the barley was aerated (2 liter sterile air per minute);
- steeping was carried out by immersion using the following scheme:

	Temperature ($^{\circ}\text{C}$)	Duration (h)
First wet stage	28	10:00
First air rest stage	20	4:00
Second wet stage	28	10:00
Second air rest stage	20	4:00
Third wet stage	28	10:00
Third air rest stage	20	4:00

Addition of the Microbial Cultures

- during steeping, 10^4 activated spores (1) per gram air dry sorghum were inoculated to the water of the first wet stage (D7).

Germination

- germination of \pm 460 g steeped sorghum was carried out in a cylindrical container with perforated lids at a temperature of 28°C during 4 days;
- air was supplied by natural diffusion;
- the containers were slowly rotated on an electronically controlled roller system (Cellroll®, Tecnorama); i.e., every two hours the containers were rolled for 15 min. at 1 rpm.

Kilning

As described in Example 1.

4. Methods of Analysis and Results

These were as described in Example 1 (4. Methods of Analysis and Results).

	Traditional Malting Process (A7)	Malting Process According to the Invention (D7)
β -glucanase Activity	98	991
Xylanase Activity	524.72	413.43

What is claimed is:

1. A process for the preparation of a malted cereal comprising the step of introducing an activated spore before or during a malting process.
2. The process according to Claim 1, wherein said activated spore increases an activity of an enzyme during said malting process.
3. The process according to Claim 1 or Claim 2, wherein said enzyme is selected from the group of β -glucanase, xylanase, amylase, a protease, naturally occurring enzymes in the cereal and combinations thereof.
4. A process for the preparation of a malted cereal as recited in claim 2 wherein the cereal, water and activated spores are combined to form a combination and where the concentration of the activated spores and the combination is held together for a time and temperature which are effective for providing the malted cereal with an enzyme activity which is greater than the enzyme activity which is obtained by a matter process without activated spores.
5. A process as recited in claim 4 wherein the combination is held for a time and temperature until the cereal has a moisture content of at least about 20 weight percent.
6. A process as recited in claims 4 or 5 wherein the combination is held until the cereal germinates and after germination, cereal is dried to a moisture content of not more than about 15 weight percent.
7. A process as recited in claim 6 wherein the combination is held until the cereal has a moisture content of between about 20 to about 60 weight percent

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9. A process as recited in claims 1, 2 or 4 wherein the activated spores are from the microbes selected from the group comprising of *Micrococcus* spp., *Streptococcus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Pediococcus halophilus*, *Pediococcus cerevisiae*, *Pediococcus damnosus*, *Pediococcus hemophilus*, *Pediococcus parvulus*, *Pediococcus soyae*, *Lactococcus* spp., *Lactobacillus* spp., *Lactobacillus acidophilus*, *Lactobacillus amylovorus*, *Lactobacillus bavaricus*, *Lactobacillus bif fermentans*, *Lactobacillus brevis* var *lindneri*, *Lactobacillus casei* var *casei*, *Lactobacillus delbrueckii*, *Lactobacillus delbrueckii* var *lactis*, *Lactobacillus delbrueckii* var *bulgaricus*, *Lactobacillus fermenti*, *Lactobacillus gasserii*, *Lactobacillus helveticus*, *Lactobacillus hilgardii*, *Lactobacillus reuteri*, *Lactobacillus sake*, *Lactobacillus sativorus*, *Lactobacillus cremoris*, *Lactobacillus kefir*, *Lactobacillus pentoceticus*, *Lactobacillus cellobiosus*, *Lactobacillus bruxellensis*, *Lactobacillus buchneri*, *Lactobacillus coryneformis*, *Lactobacillus confusus*, *Lactobacillus florentinus*, *Lactobacillus viridescens*, *Corynebacterium* spp., *Propionibacterium* spp., *Bifidobacterium* spp., *Streptomyces* spp., *Bacillus* spp., *Sporolactobacillus* spp., *Acetobacter* spp., *Agrobacterium* spp., *Alcaligenes* spp., *Pseudomonas* spp., *Pseudomonas amylophila*, *Pseudomonas aeruginosa*, *Pseudomonas cocovenenans*, *Pseudomonas mexicana*, *Pseudomonas pseudomallei*, *Gluconobacter* spp., *Enterobacter* spp., *Erwinia* spp., *Klebsiella* spp., *Proteus* spp., *Ascomycota*

Dothideales, Mycosphaerellaceae, Mycosphaerella spp., Venturiaceae, Venturia spp., Eurotiales, Monascaceae, Monascus spp., Trichocomaceae, Emericella spp., Eurotium spp., Eupenicillium spp., Neosartorya spp., Talaromyces spp., Hypocreales, Hypocreaceae, Hypocrea spp., Saccharomycetales, Dipodascaceae, Dipodascus spp., Galactomyces spp., Endomycetaceae, Endomyces spp., Metschnikowiaceae, Guilliermondella spp., Saccharomycetaceae, Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccharomycodaceae, Hanseniaspora spp.; Schizosaccharomycetales, Schizosaccharomycetaceae, Schizosaccharomyces spp., Sordariales, Chaetomiaceae, Chaetomium spp., Sordariaceae, Neurospora spp., Zygomycota, Mucorales, Mucoraceae, Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Chlamydomucor spp., Mucor spp., Mucor circinelloides, Mucor grisecyanus, Mucor hiemalis, Mucor indicus, Mucor mucedo, Mucor piriformis, Mucor plumbeus, Mucor praini, Mucor pusillus, Mucor silvaticus, Mucor javanicus, Mucor racemosus, Mucor rouxianus, Mucor rouxii, Mucor aromaticus, Mucor flavus, Mucor miehei, Rhizopus spp., Rhizopus arrhizus, Rhizopus oligosporus, Rhizopus oryzae, Rhizopus oryzae strain ATCC 4858, Rhizopus oryzae strain ATCC 9363, Rhizopus oryzae strain NRRL 1891, Rhizopus oryzae strain NRRL 1472, Rhizopus stolonifer, Rhizopus thailandensis, Rhizopus formosensis, Rhizopus chinensis, Rhizopus cohnii, Rhizopus japonicus, Rhizopus nodosus, Rhizopus delemar, Rhizopus acetorinus, Rhizopus chlamydosporus, Rhizopus circinans, Rhizopus javanicus, Rhizopus peka, Rhizopus saito, Rhizopus tritici, Rhizopus niveus, Rhizopus microsporus, Mitosporic fungi, Aureobasidium spp., Acremonium spp., Cercospora spp., Epicoccum spp., Monilia spp., Monilia candida, Monilia sitophila, Mycoderma spp., Candida spp., Candida diddensiae, Candida edax, Candida etchellsii, Candida kefir, Candida krisei, Candida lactosa, Candida lambica, Candida melinii, Candida utilis, Candida milleri, Candida mycoderma, Candida parapsilosis, Candida obtux, Candida tropicalis, Candida

valida, *Candida versatilis*, *Candida guilliermondii*, *Rhodotorula* spp., *Torulopsis* spp., *Geotrichum* spp., *Geotrichum amycellum*, *Geotrichum amillariae*, *Geotrichum asteroides*, *Geotrichum bipunctatum*, *Geotrichum dulcitum*, *Geotrichum erlense*, *Geotrichum fici*, *Geotrichum flavo-brunneum*, *Geotrichum fragrans*, *Geotrichum gracile*, *Geotrichum hirtum*, *Geotrichum klebakii*, *Geotrichum penicillatum*, *Geotrichum hirtum*, *Geotrichum pseudocandidum*, *Geotrichum rectangulatum*, *Geotrichum suaveolens*, *Geotrichum vannyiae*, *Geotrichum loubieri*, *Geotrichum microsporum*, *Cladosporium* spp., *Trichoderma* spp., *Trichoderma hamatum*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma pseudokoningii*, *Trichoderma reesei*, *Trichoderma virgatum*, *Trichoderma viride*, *Oldium* spp., *Alternaria* spp., *Alternaria alternata*, *Alternaria tenuis*, *Helminthosporium* spp., *Helminthosporium gramineum*, *Helminthosporium sativum*, *Helminthosporium teres*, *Aspergillus* spp., *Aspergillus ochraceus*, *Aspergillus nidulans*, *Aspergillus versicolor*, *Aspergillus wentii* Group, *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus oryzae* strain ATCC 14156, *Penicillium* spp., *Penicillium aculeatum*, *Penicillium citrinum*, *Penicillium claviforme*, *Penicillium funiculosum*, *Penicillium italicum*, *Penicillium lanoso-viride*, *Penicillium emersonii*, *Penicillium lilacinum*, *Penicillium expansum* and mixtures thereof.

10. A malted cereal product made according to the process of claims 1 through 9.

11. An aqueous combination of a cereal and activated spores.

12. A process as recited in claim 11 wherein the activated spores are from the microbes selected from the group comprising *Micrococcus* spp., *Streptococcus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Pediococcus halophilus*, *Pediococcus cerevisiae*, *Pediococcus damnosus*, *Pediococcus*

hemophilus, *Pediococcus parvulus*, *Pediococcus soyae*, *Lactococcus* spp., *Lactobacillus* spp., *Lactobacillus acidophilus*, *Lactobacillus amylovorus*, *Lactobacillus bavaricus*, *Lactobacillus bifermens*, *Lactobacillus brevis* var *lindneri*, *Lactobacillus casei* var *casei*, *Lactobacillus delbrueckii*, *Lactobacillus delbrueckii* var *lactis*, *Lactobacillus delbrueckii* var *bulgaricus*, *Lactobacillus fermenti*, *Lactobacillus gasserii*, *Lactobacillus helveticus*, *Lactobacillus hilgardii*, *Lactobacillus reuteri*, *Lactobacillus sake*, *Lactobacillus sativorus*, *Lactobacillus cremoris*, *Lactobacillus kefir*, *Lactobacillus pentoceticus*, *Lactobacillus cellobiosus*, *Lactobacillus bruxellensis*, *Lactobacillus buchnerii*, *Lactobacillus coryneformis*, *Lactobacillus confusus*, *Lactobacillus florentinus*, *Lactobacillus viridescens*, *Corynebacterium* spp., *Propionibacterium* spp., *Bifidobacterium* spp., *Streptomyces* spp., *Bacillus* spp., *Sporolactobacillus* spp., *Acetobacter* spp., *Agrobacterium* spp., *Alcaligenes* spp., *Pseudomonas* spp., *Pseudomonas amylophila*, *Pseudomonas aeruginosa*, *Pseudomonas cocovenenans*, *Pseudomonas mexicana*, *Pseudomonas pseudomallei*, *Gluconobacter* spp., *Enterobacter* spp., *Erwinia* spp., *Klebsiella* spp., *Proteus* spp., Ascomycota, Dothideales, *Mycosphaerellaceae*, *Mycosphaerella* spp., *Venturiaceae*, *Venturia* spp., Eurotiales, *Monascaceae*, *Monascus* spp., *Trichocomaceae*, *Emmericella* spp., *Eurotium* spp., *Eupenicillium* spp., *Neosartorya* spp., *Talaromyces* spp., Hypocreales, *Hypocreaceae*, *Hypocrea* spp., *Saccharomycetales*, *Dipodascaceae*, *Dipodascus* spp., *Galactomyces* spp., *Endomycetaceae*, *Endomyces* spp., *Metschnikowiaceae*, *Gulliermondella* spp., *Saccharomycetaceae*, *Debaryomyces* spp., *Dekkera* spp., *Pichia* spp., *Kluyveromyces* spp., *Saccharomyces* spp., *Torulaspora* spp., *Zygosaccharomyces* spp., *Saccharomycodaceae*, *Hanseniaspora* spp.; *Schizosaccharomycetales*, *Schizosaccharomycetaceae*, *Schizosaccharomyces* spp., Sordariales, *Chaetomiaceae*, *Chaetomium* spp., *Sordariaceae*, *Neurospora* spp., Zygomycota, *Mucorales*, *Mucoraceae*, *Absidia* spp., *Amylomyces* spp., *Rhizomucor* spp., *Actinomucor* spp., *Thermomucor* spp., *Chlamydomucor* spp., *Mucor* spp., *Mucor circinelloides*, *Mucor grisecyanus*,

Mucor hiemalis, *Mucor indicus*, *Mucor mucedo*, *Mucor piriformis*, *Mucor plumbeus*, *Mucor praini*, *Mucor pusillus*, *Mucor silvaticus*, *Mucor javanicus*, *Mucor racemosus*, *Mucor rouxianus*, *Mucor rouxii*, *Mucor aromaticus*, *Mucor flavus*, *Mucor miehei*, *Rhizopus* spp., *Rhizopus arrhizus*, *Rhizopus oligosporus*, *Rhizopus oryzae*, *Rhizopus oryzae* strain ATCC 4858, *Rhizopus oryzae* strain ATCC 9363, *Rhizopus oryzae* strain NRRL 1891, *Rhizopus oryzae* strain NRRL 1472, *Rhizopus stolonifer*, *Rhizopus thailandensis*, *Rhizopus formosensis*, *Rhizopus chinensis*, *Rhizopus cohnii*, *Rhizopus japonicus*, *Rhizopus nodosus*, *Rhizopus delemar*, *Rhizopus acetorinus*, *Rhizopus chlamydosporus*, *Rhizopus circinans*, *Rhizopus javanicus*, *Rhizopus peka*, *Rhizopus salto*, *Rhizopus tritici*, *Rhizopus niveus*, *Rhizopus microsporus*, *Mitosporic fungi*, *Aureobasidium* spp., *Acremonium* spp., *Cercospora* spp., *Epicoccum* spp., *Monilia* spp., *Monilia candida*, *Monilia sitophila*, *Mycoderma* spp., *Candida* spp., *Candida diddensiae*, *Candida edax*, *Candida etchellsii*, *Candida kefir*, *Candida krisei*, *Candida lactosa*, *Candida lambica*, *Candida melinii*, *Candida utilis*, *Candida milleri*, *Candida mycoderma*, *Candida parapsilosis*, *Candida obtux*, *Candida tropicalis*, *Candida valida*, *Candida versatilis*, *Candida guilliermondii*, *Rhodotorula* spp., *Torulopsis* spp., *Geotrichum* spp., *Geotrichum amycelium*, *Geotrichum armillariae*, *Geotrichum asteroides*, *Geotrichum bipunctatum*, *Geotrichum dulcitum*, *Geotrichum ericense*, *Geotrichum fici*, *Geotrichum flavo-brunneum*, *Geotrichum fragrans*, *Geotrichum gracile*, *Geotrichum hirtum*, *Geotrichum klebaknii*, *Geotrichum penicillatum*, *Geotrichum hirtum*, *Geotrichum pseudocandidum*, *Geotrichum rectangulatum*, *Geotrichum suaveolens*, *Geotrichum vanryiae*, *Geotrichum loubieri*, *Geotrichum microsporum*, *Cladosporium* spp., *Trichoderma* spp., *Trichoderma hamatum*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma pseudokoningii*, *Trichoderma reesei*, *Trichoderma virgatum*, *Trichoderma viride*, *Oidium* spp., *Alternaria* spp., *Alternaria alternata*, *Alternaria tenuis*, *Helminthosporium* spp., *Helminthosporium gramineum*, *Helminthosporium sativum*, *Helminthosporium teres*, *Aspergillus* spp., *Aspergillus*

13. A process for the preparation of a malted cereal said process comprising the steps of:

- (a) introducing an activated spore into a moistened cereal to provide an inoculated moistened cereal to form a moistened cereal/activated spore combination;
- (b) germinating said inoculated moistened cereal; and
- (c) drying said germinated cereal.

14. The process according to Claim 13, wherein said inoculated moistened cereal is held at a temperature of from about 5° to about 30°C until the cereal has a moisture content of from about 20 to about 60 weight percent moisture.

15. The process according to Claim 13 or Claim 14, wherein said germinating step (b) is carried out for about 3 to about 6 days at a temperature of from about 10° to about 30°C.

16. The process according to any one of Claims 13 to 15, wherein said germinated cereal is dried to a moisture content of from about 2 to about 15 weight percent.

17. A process as recited in claim 13 wherein the combination is held at a temperature of from about 10°C to about 20°C until the cereal has a moisture content of from about 38 to about 47 weight percent and the cereal has germinated for about 3 to about 6 days at a temperature of from about 14°C to about 18°C and the germinated cereal is dried at a temperature of from about 40°C to about 150°C.

18. A process for the preparation of a malted cereal said process comprising the step of moistening a cereal and activated spores wherein the concentration of the activated spores, moistening time and moistening temperature are effective for providing the malted cereal with an increase in activity of an enzyme compared to the activity of an enzyme obtained by moistening the cereal without activated spores.

19. The process according to Claim 18, wherein said enzyme is selected from the group of β -glucanase, xylanase, amylase, protease, naturally occurring enzymes in the cereal and combinations thereof.

20. A process as recited in claim 18 wherein the cereal moistening time and temperature are effective to provide the cereal with a moisture content of at least about 20 weight percent.

21. A process as recited in claim 20 wherein after cereal attains a moisture content of at least about 20 weight percent, it is dried to a moisture content of not more than about 15 weight percent.

22. A process as recited in claims 18, 19, 20 or 21 wherein the moistening time and temperature are effective to provide the cereal with a moisture content of between about 20 to about 60 weight percent and wherein the cereal has

24. A process as recited in claim 23 wherein the activated spores are from the microbes selected from the group comprising *Micrococcus* spp., *Streptococcus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Pediococcus halophilus*, *Pediococcus cerevisiae*, *Pediococcus damnosus*, *Pediococcus hemophilus*, *Pediococcus parvulus*, *Pediococcus soyae*, *Lactococcus* spp., *Lactobacillus* spp., *Lactobacillus acidophilus*, *Lactobacillus amylovorus*, *Lactobacillus bavaricus*, *Lactobacillus bifementans*, *Lactobacillus brevis* var *lindneri*, *Lactobacillus casei* var *casei*, *Lactobacillus delbrueckii*, *Lactobacillus delbrueckii* var *lactis*, *Lactobacillus delbrueckii* var *bulgaricus*, *Lactobacillus fermenti*, *Lactobacillus gasserii*, *Lactobacillus helveticus*, *Lactobacillus hilgardii*, *Lactobacillus reuteri*, *Lactobacillus sake*, *Lactobacillus sativorus*, *Lactobacillus cremoris*, *Lactobacillus kefir*, *Lactobacillus pentoceticus*, *Lactobacillus cellobiosus*, *Lactobacillus bruxellensis*, *Lactobacillus buchnerii*, *Lactobacillus coryneformis*, *Lactobacillus confusus*, *Lactobacillus florentinus*, *Lactobacillus viridescens*, *Corynebacterium* spp., *Propionibacterium* spp., *Bifidobacterium* spp., *Streptomyces* spp., *Bacillus* spp., *Sporolactobacillus* spp., *Acetobacter* spp., *Agrobacterium* spp., *Alcaligenes* spp., *Pseudomonas* spp., *Pseudomonas amylophila*, *Pseudomonas aeruginosa*, *Pseudomonas cocovenenans*, *Pseudomonas mexicana*, *Pseudomonas pseudomallei*, *Gluconobacter* spp., *Enterobacter* spp., *Erwinia* spp., *Klebsiella* spp., *Proteus* spp., *Ascomycota*, *Dothideales*, *Mycosphaerellaceae*, *Mycosphaerella* spp., *Venturiaceae*, *Venturia* spp., *Eurotiales*, *Monascaceae*, *Monascus* spp., *Trichocomaceae*, *Emmericilia* spp., *Euroteum* spp., *Eupenicillium* spp., *Neosartorya* spp., *Talaromyces* spp.,

Hypocreales, Hypocreaceae, Hypocrea spp., Saccharomycetales, Dipodascaceae, Dipodascus spp., Galactomyces spp., Endomycetaceae, Endomyces spp., Metschnikowiaceae, Guilliermondella spp., Saccharomycetaceae, Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccharomycodaceae, Hanseniaspora spp.; Schizosaccharomycetales, Schizosaccharomycetaceae, Schizosaccharomyces spp., Sordariales, Chaetomiaceae, Chaetomium spp., Sordariaceae, Neurospora spp., Zygomycota, Mucorales, Mucoraceae, Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Chlamydomucor spp., Mucor spp., Mucor circinelloides, Mucor grisecyanus, Mucor hiemalis, Mucor indicus, Mucor mucedo, Mucor piriformis, Mucor plumbeus, Mucor praini, Mucor pusillus, Mucor silvaticus, Mucor javanicus, Mucor racemosus, Mucor rouxianus, Mucor rouxii, Mucor aromaticus, Mucor flavus, Mucor miehei, Rhizopus spp., Rhizopus arrizus, Rhizopus oligosporus, Rhizopus oryzae, Rhizopus oryzae strain ATCC 4858, Rhizopus oryzae strain ATCC 9363, Rhizopus oryzae strain NRRL 1891, Rhizopus oryzae strain NRRL 1472, Rhizopus stolonifer, Rhizopus thailandensis, Rhizopus formosensis, Rhizopus chinensis, Rhizopus cohnii, Rhizopus japonicus, Rhizopus nodosus, Rhizopus delemar, Rhizopus acetorinus, Rhizopus chlamydosporus, Rhizopus circinans, Rhizopus javanicus, Rhizopus peka, Rhizopus saito, Rhizopus tritici, Rhizopus niveus, Rhizopus microsporus, Mitosporic fungi, Aureobasidium spp., Acremonium spp., Cercospora spp., Epicoccum spp., Monilia spp., Monilia candida, Monilia sitophila, Mycoderma spp., Candida spp., Candida diddensiae, Candida edax, Candida etchellsii, Candida kefir, Candida krusei, Candida lactosa, Candida lambica, Candida melinii, Candida utilis, Candida milleri, Candida mycoderma, Candida parapsilosis, Candida obtusa, Candida tropicalis, Candida valida, Candida versatilis, Candida guilliermondii, Rhodotorula spp., Torulopsis spp., Geotrichum spp., Geotrichum amycellum, Geotrichum armillariae, Geotrichum asteroides, Geotrichum bipunctatum, Geotrichum dulcium,

Geotrichum eriense, *Geotrichum fici*, *Geotrichum flavo-brunneum*, *Geotrichum fragrans*, *Geotrichum gracile*, *Geotrichum heritum*, *Geotrichum klebaknii*, *Geotrichum penicillatum*, *Geotrichum hirtum*, *Geotrichum pseudocandidum*, *Geotrichum rectangulatum*, *Geotrichum suaveolens*, *Geotrichum vanrylae*, *Geotrichum loubieri*, *Geotrichum microsporum*, *Cladosporium* spp., *Trichoderma* spp., *Trichoderma hamatum*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma pseudokoningii*, *Trichoderma reesei*, *Trichoderma virgatum*, *Trichoderma viride*, *Oidium* spp., *Alternaria* spp., *Alternaria alternata*, *Alternaria tenuis*, *Helminthosporium* spp., *Helminthosporium gramineum*, *Helminthosporium sativum*, *Helminthosporium teres*, *Aspergillus* spp., *Aspergillus ochraseus*, *Aspergillus nidulans*, *Aspergillus versicolor*, *Aspergillus wentii* Group, *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus oryzae* strain ATCC 14156, *Penicillium* spp., *Penicillium aculeatum*, *Penicillium citrinum*, *Penicillium claviforme*, *Penicillium funiculosum*, *Penicillium italicum*, *Penicillium lanoso-viride*, *Penicillium emersonii*, *Penicillium lilacinum*, *Penicillium expansum* and mixtures thereof.

25. A malted cereal product made according to the process of claims 18, 19, 20, 21, 22, 23 or 24.

26. Use of activated spores in the preparation of a malted cereal.

27. A process for the preparation of malted cereals, wherein the steeping step includes one or more wetting stages at a temperature between 5° and 30° C, preferably between 10° and 20° C, until the material has a moisture content between 20% and 60% by weight, preferably between 38% and 47%, wherein after a germination period between 2 and 7 days, preferably between 3 to 6 days at a temperature between 10° and 30° C, preferably between 14° and 18° C, the steeped and germinated cereals are preferably kilned by increasing

the temperature to values between 40° and 150° C until the material has a moisture content between 2% and 15% by weight, and wherein one or more microbial cultures selected from the group consisting of one or more bacteria and/or one or more fungi are added in one or more times either before or during or after the malting process of said cereals.

28. Process according to claim 27, for the preparation of malted barley, wherein the bacteria are selected from the group comprising *Micrococcus* spp., *Streptococcus* spp., *Leuconostoc* spp., *Pediococcus* spp. preferentially *Pediococcus halophilus*, *Pediococcus cerevisiae*, *Pediococcus damnosus*, *Pediococcus hemophilus*, *Pediococcus parvulus*, *Pediococcus soya*, *Lactococcus* spp., *Lactobacillus* spp. preferentially *Lactobacillus acidophilus*, *Lactobacillus amylovorus*, *Lactobacillus bavaricus*, *Lactobacillus bif fermentans*, *Lactobacillus brevis* var *lindneri*, *Lactobacillus casei* var *casei*, *Lactobacillus delbrueckii*, *Lactobacillus delbrueckii* var *lactis*, *Lactobacillus delbrueckii* var *bulgaricus*, *Lactobacillus fermenti*, *Lactobacillus gasserii*, *Lactobacillus helveticus*, *Lactobacillus hilgardii*, *Lactobacillus reuteri*, *Lactobacillus sake*, *Lactobacillus sativarius*, *Lactobacillus cremoris*, *Lactobacillus kefir*, *Lactobacillus pentoceticus*, *Lactobacillus cellobiosus*, *Lactobacillus bruxellensis*, *Lactobacillus buchnerii*, *Lactobacillus coryneformis*, *Lactobacillus confusus*, *Lactobacillus florentinus*, *Lactobacillus viridescens*, *Corynebacterium* spp., *Propionibacterium* spp., *Bifidobacterium* spp., *Streptomyces* spp., *Bacillus* spp., *Sporolactobacillus* spp., *Acetobacter* spp., *Agrobacterium* spp., *Alcaligenes* spp., *Pseudomonas* spp. preferentially *Pseudomonas amylophilia*, *Pseudomonas aeruginosa*, *Pseudomonas cocovenenana*, *Pseudomonas mexicana*, *Pseudomonas pseudomallei*, *Gluconobacter* spp., *Enterobacter* spp., *Erwinia* spp., *Klebsiella* spp., and *Proteus* spp.

29. The process according to claim 27, for the preparation of malted barley wherein the fungi are selected from the group (genera as described by Ainsworth and Bisby's dictionary of the fungi, 8th edition, 1995, edited by D.L. Hawksworth, P.M. Kirk, B.C. Sutton, and D.N. Pegler (632 pp) Cab International) comprising Ascomycota preferentially Dothideales preferentially Mycosphaerellaceae preferentially Mycosphaerella spp., Venturiaceae preferentially Venturia spp.; Eurotiales preferentially Monascaceae preferentially Monascus spp., Trichocomaceae preferentially Emericella spp., Eurotium spp., Eupenicillium spp., Neosartorya spp., Talaromyces spp.; Hypocreales preferentially Hypocreaceae preferentially Hypocrea spp.; Saccharomycetales preferentially Dipodascaceae preferentially Dipodascus spp., Galactomyces spp., Endomycetaceae preferentially Endomyces spp., Metschnikowiaceae preferentially Guilliermondella spp., Saccharomycetaceae preferentially Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccharomycodaceae preferentially Hanseniaspora spp.; Schizosaccharomycetales preferentially Schizosaccharomycetaceae preferentially Schizosaccharomyces spp.; Sordariales preferentially Chaetomiaceae preferentially Chaetomium spp., Sordariaceae preferentially Neurospora spp.; Zygomycota preferentially Mucorales preferentially Mucoraceae preferentially Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Chlamydomucor spp., Mucor spp. preferentially Mucor circinelloides, Mucor griseocyanus, Mucor hiemalis, Mucor indicus, Mucor mucedo, Mucor piriformis, Mucor plumbeus, Mucor praini, Mucor pusillus, Mucor silvaticus, Mucor javanicus, Mucor racemosus, Mucor rouxianus, Mucor rouxii, Mucor aromaticus, Mucor flavus, Mucor miehei, Rhizopus spp. preferentially Rhizopus arrizus, Rhizopus oligosporus, Rhizopus oryzae preferentially strains ATCC 4858, ATCC 9363, NRRL 1891, NRRL 1472, Rhizopus stolonifer, Rhizopus thailandensis, Rhizopus formosensis, Rhizopus

chinensis, *Rhizopus cohnii*, *Rhizopus japonicus*, *Rhizopus nodosus*, *Rhizopus delemar*, *Rhizopus acetorinus*, *Rhizopus chlamydosporus*, *Rhizopus circinans*, *Rhizopus javanicus*, *Rhizopus peka*, *Rhizopus saito*, *Rhizopus tritici*, *Rhizopus niveus*, *Rhizopus microsporus*; Mitosporic fungi preferentially *Aureobasidium* spp., *Acremonium* spp., *Cercospora* spp., *Epicoccum* spp., *Monilia* spp. preferentially *Monilia candida*, *Monilia strophila*, *Mycoderma* spp., *Candida* spp. preferentially *Candida diddensiae*, *Candida edax*, *Candida etchellsii*, *Candida kefir*, *Candida krisei*, *Candida lactosa*, *Candida lambica*, *Candida melinii*, *Candida utilis*, *Candida milleri*, *Candida mycoderma*, *Candida parapsilosis*, *Candida obtux*, *Candida tropicalis*, *Candida valida*, *Candida versatilis*, *Candida guilliermondii*, *Rhodotorula* spp., *Torulopsis* spp., *Geotrichum* spp. preferentially *Geotrichum amycelium*, *Geotrichum armillariae*, *Geotrichum asteroides*, *Geotrichum bipunctatum*, *Geotrichum dulcitum*, *Geotrichum ericense*, *Geotrichum fici*, *Geotrichum flavo-brunneum*, *Geotrichum fragrans*, *Geotrichum gracile*, *Geotrichum heritum*, *Geotrichum klebaknii*, *Geotrichum penicillatum*, *Geotrichum hirtum*, *Geotrichum pseudocandidum*, *Geotrichum rectangulatum*, *Geotrichum suaveolens*, *Geotrichum vanryiae*, *Geotrichum loubieri*, *Geotrichum microsporum*, *Cladosporium* spp., *Trichoderma* spp. preferentially *Trichoderma hamatum*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma pseudokoningii*, *Trichoderma reesei*, *Trichoderma virgatum*, *Trichoderma viride*, *Oidium* spp., *Alternaria* spp. preferentially *Alternaria alternata*, *Alternaria tenuis*, *Helminthosporium* spp. preferentially *Helminthosporium gramineum*, *Helminthosporium sativum*, *Helminthosporium teres*, *Aspergillus* spp. as described by R.A. Samson ((1994) in *Biotechnological handbooks*, Volume 7: *Aspergillus*, edited by Smith, J.E. (273 pp), Plenum Press) preferentially *Aspergillus ochraceus* Group (Thom & Church), *Aspergillus nidulans* Group (Thom & Church), *Aspergillus versicolor* Group (Thom & Church), *Aspergillus wentii* Group (Thom & Raper), *Aspergillus candidus* Group (Thom & Raper), *Aspergillus flavus* Group (Raper & Fennell), *Aspergillus niger* Group (Thom &

Church), *Penicillium* spp. preferentially *Penicillium aculeatum*, *Penicillium citrinum*, *Penicillium claviforme*, *Penicillium funiculosum*, *Penicillium italicum*, *Penicillium lanoso-viride*, *Penicillium emersonii*, *Penicillium lilacinum*, and *Penicillium expansum*.

30. The process according to claim 27 for the preparation of malted cereals other than malted barley wherein the bacteria are selected from the group comprising *Micrococcus* spp., *Streptococcus* spp., *Leuconostoc* spp., *Pediococcus* spp., *Lactococcus* spp., *Lactobacillus* spp., *Corynebacterium* spp., *Propionibacterium* spp., *Bifidobacterium* spp., *Streptomyces* spp., *Bacillus* spp., *Sporolactobacillus* spp., *Acetobacter* spp., *Agrobacterium* spp., *Alcaligenes* spp., *Pseudomonas* spp., *Gluconobacter* spp., *Enterobacter* spp., *Erwinia* spp., *Klebsiella* spp., and *Proteus* spp.

31. Process according to claim 27 for the preparation of malted cereals other than malted barley wherein the fungi are selected from the group comprising Ascomycota preferentially Dothideales preferentially Mycosphaerellaceae preferentially Mycosphaerella spp., Venturiaceae preferentially Venturia spp.; Eurotiales preferentially Monascaceae preferentially Monascus spp., Trichocomaceae preferentially Emericella spp., Eurotium spp., Eupenicillium spp., Neosartorya spp., Talaromyces spp.; Hypocreales preferentially Hypocreaceae preferentially Hypocrea spp.; Saccharomycetales preferentially Dipodascaceae preferentially Dipodascus spp., Galactomyces spp., Endomycetaceae preferentially Endomyces spp., Metschnikowiaceae preferentially Guilliermondella spp., Saccharomycetaceae preferentially Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccharomycodaceae preferentially Hanseniaspora spp.; Schizosaccharomycetales preferentially Schizosaccharomycetaceae

preferentially *Schizosaccharomyces* spp.; Sordariales preferentially Chaetomiaceae preferentially *Chaetomium* spp., Sordariaceae preferentially *Neurospora* spp.; Zygomycota preferentially Mucorales preferentially Mucoraceae preferentially *Absidia* spp., *Amylomyces* spp., *Rhizomucor* spp., *Actinomucor* spp., *Thermomucor* spp., *Chlamydomucor* spp., *Mucor* spp., *Rhizopus* spp.; Mitosporic fungi preferentially *Aureobasidium* spp., *Acremonium* spp., *Cercoospora* spp., *Epicoccum* spp., *Monilia* spp., *Mycoderma* spp., *Candida* spp., *Rhodotorula* spp., *Torulopsis* spp., *Geotrichum* spp., *Cladosporium* spp., *Trichoderma* spp., *Oidium* spp., *Alternaria* spp., *Helminthosporium* spp., *Aspergillus* spp., and *Penicillium* spp.

32. Process according to any of Claims 27 to 31, wherein the total time of submersion in water during steeping for physiological reasons does not exceed 30 hours, preferentially takes 10 to 25 hours, or wherein the kilning includes more than two temperature steps and wherein the microbial culture comprises *Rhizopus* spp. and/or *Pseudomonas* spp.

33. Process according to the claim 32, wherein the *Rhizopus* spp. is preferably a *Rhizopus oryzae* such as a *Rhizopus oryzae* strain ATCC 9363.

34. Process according to the claim 31 or claim 32, wherein the *Pseudomonas* sp. is preferably a *Pseudomonas herbicola*.

35. Process according to any of claims 27 to 35, wherein the microbial spores used are activated by one or a combination of the following treatments:

- (a) cycles of wetting and/or drying,
- (b) addition of nutritional supplies or addition of spore elements.
- (c) exposure to temperature changes, preferably within a range of 0° to 80° C,

- (d) exposure to changes in pH, preferably within a pH range of 2.0 to 8.0, more preferably between 3.0 and 6.0, to obtain spores significantly more swollen than their dormant size, more particularly, the size of the spores is increased by a factor preferably between 1.2 and 10 over their dormant size and/or spores with one or more germ tubes per spore.

36. Process according to any one of claims 27 to 35, wherein the pH during the steeping step is adjusted to a value between 4.0 and 6.0.

37. Process according to any one of claims 27 to 36, wherein nutrients and/or additives are added prior to and/or during the malting process.

38. Malted barley characterized by a β -glucanase activity increased by at least a factor 4 and a xylanase activity increased by at least a factor of 4, compared to the conventional malting process of any available barley.

39. Malted barley, wherein the β -glucanase activity is higher than 700 units/kg. and the xylanase activity is higher than 250 units/kg.

40. Malted barley according to claim 38 or 39 obtained by the process of any one of the claims 27 to 37.

41. Malted barley according to any one of claims 38 to 40, characterized in that they present an improved modification and/or an increased hydrolytic enzyme activity, a decreased level of toxins and/or increased microbial safety or increased acceptability.

42. Use of the malted cereals according to any one of the claims 38 to 41, or obtained by the process of any one of the claims 27 to 37 for the preparation of beverages.

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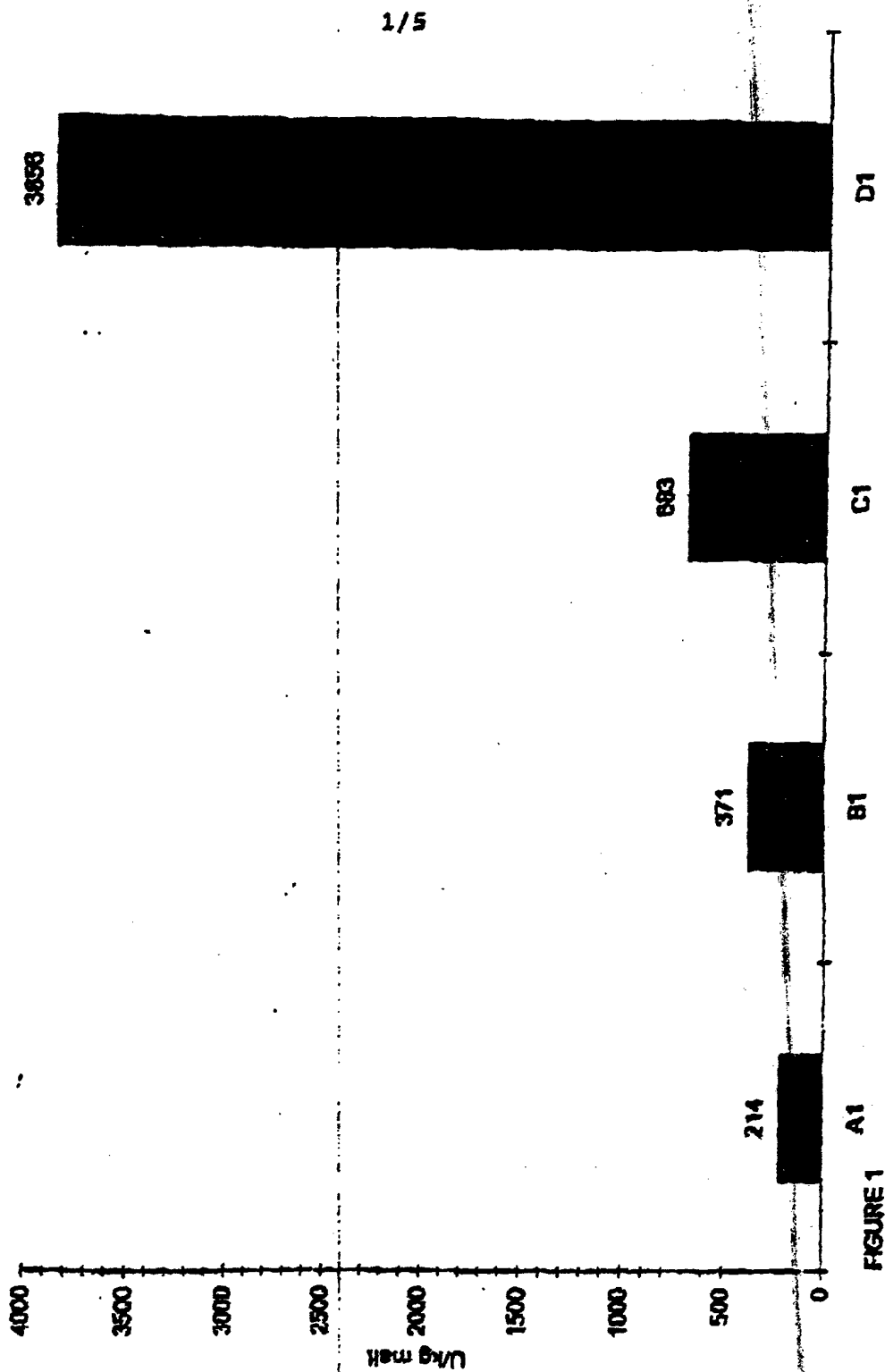
ABSTRACT

Process for the preparation of malted cereals, wherein the steeping step includes one or more wetting stages at a temperature between 5 and 30° C, preferably between 10 and 20° C, until the material has a moisture content between 20 and 60% by weight, preferably between 38 and 47%, wherein after a germination period between 2 and 7 days, preferably between 3 to 6 days at a temperature between 10 and 30° C, preferably between 14 and 18° C, the steeped and germinated cereals are preferably kilned by increasing the temperature to values between 40 and 150° C until the material has a moisture content between 2 and 15% by weight, and wherein one or more microbial cultures selected from the group consisting of one or more bacteria and/or one of more activated spores are added in one or more times during the process.

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Glucanase activity



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2/5

Xylanase activity

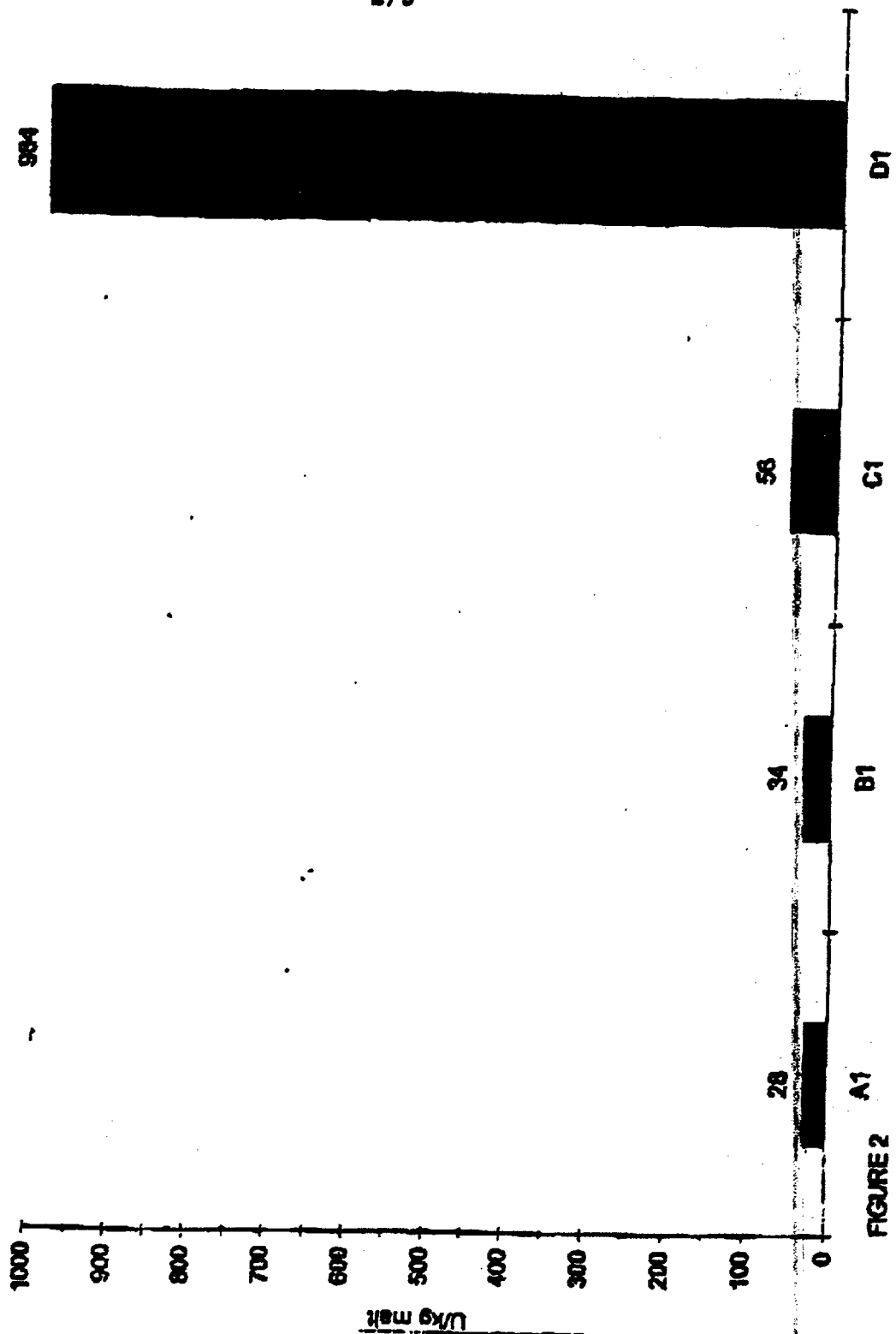


FIGURE 2

一、二、三、四、五、六、七、八、九、十、十一、十二、十三、十四、十五、十六、十七、十八、十九、二十、二十一、二十二、二十三、二十四、二十五、二十六、二十七、二十八、二十九、三十、三十一、三十二、三十三、三十四、三十五、三十六、三十七、三十八、三十九、四十、四十一、四十二、四十三、四十四、四十五、四十六、四十七、四十八、四十九、五十、五十一、五十二、五十三、五十四、五十五、五十六、五十七、五十八、五十九、六十、六十一、六十二、六十三、六十四、六十五、六十六、六十七、六十八、六十九、七十、七十一、七十二、七十三、七十四、七十五、七十六、七十七、七十八、七十九、八十、八十一、八十二、八十三、八十四、八十五、八十六、八十七、八十八、八十九、九十、九十一、九十二、九十三、九十四、九十五、九十六、九十七、九十八、九十九、一百。



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Xylanase activity

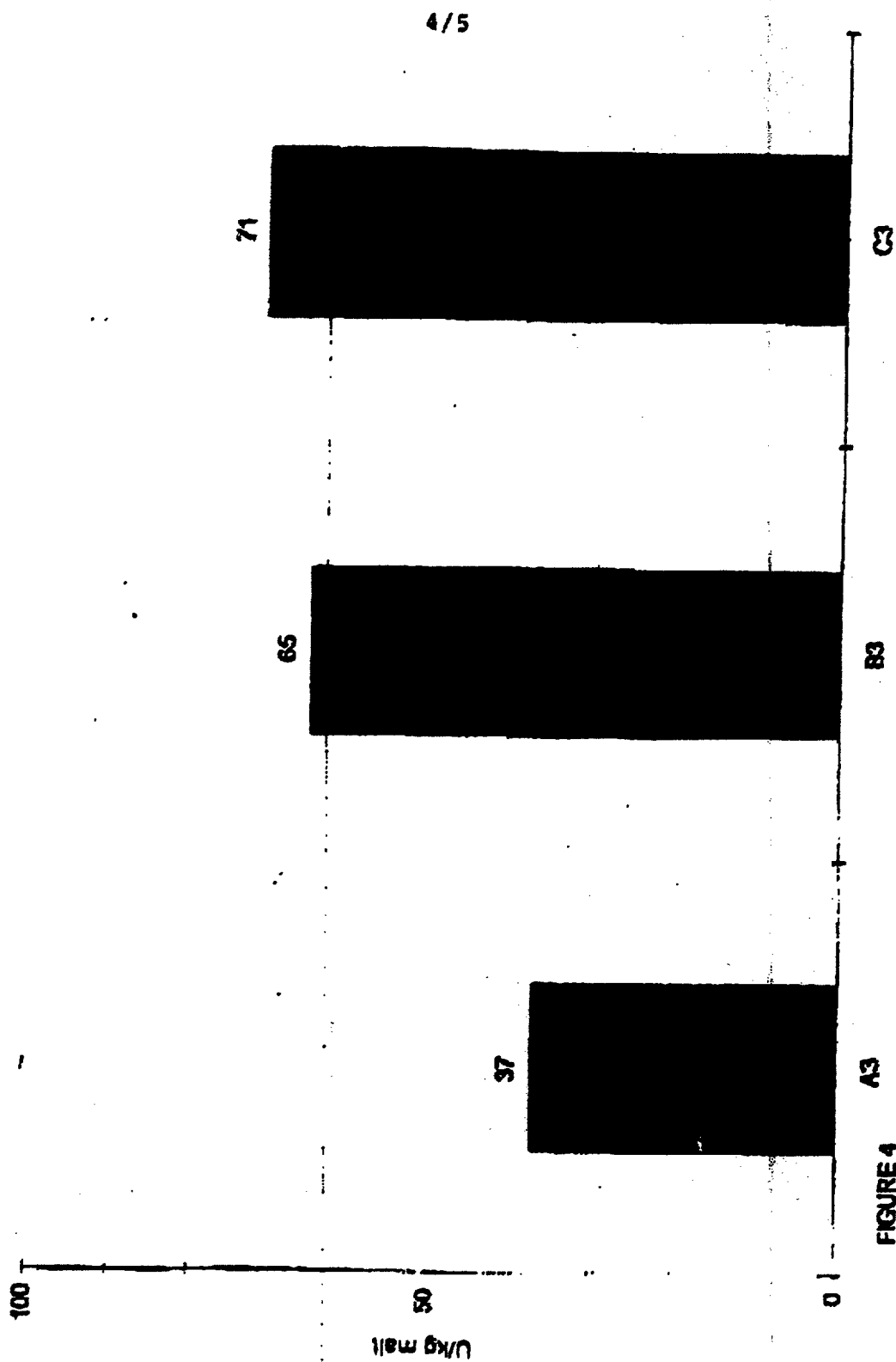


FIGURE 4

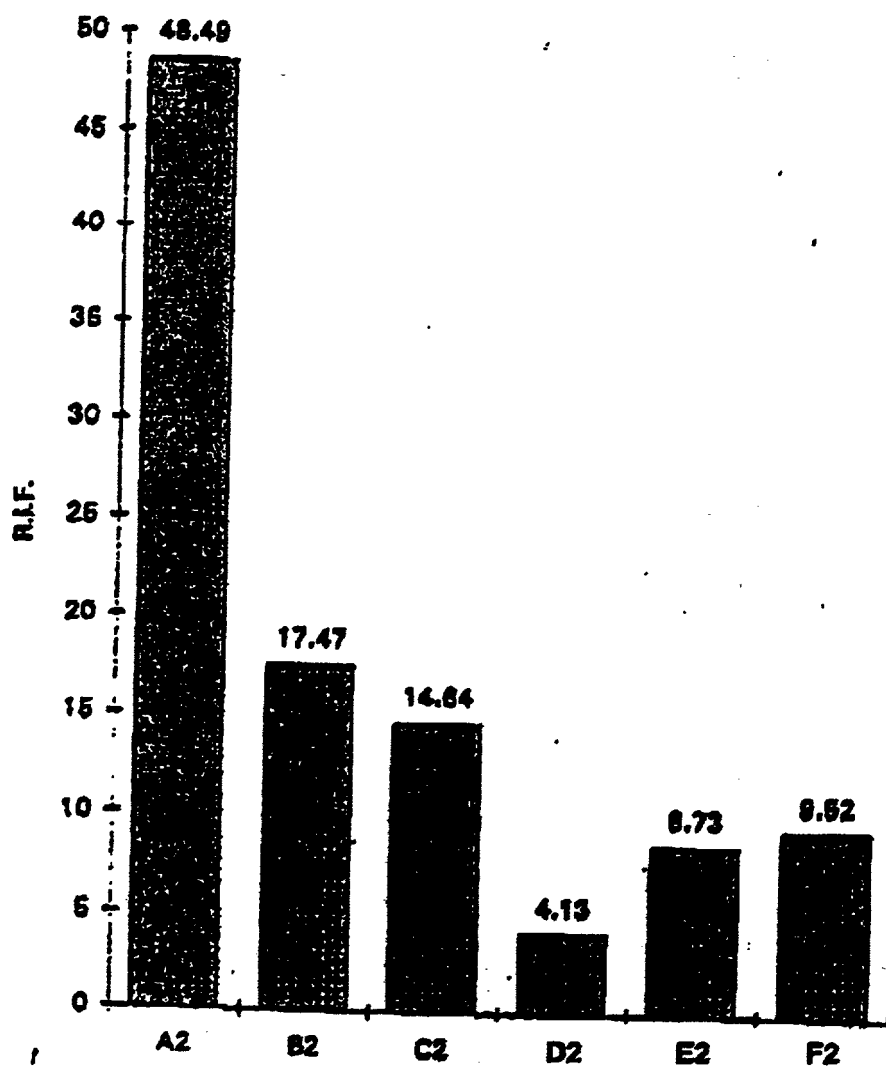


FIGURE 5

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PROCESS FOR THE PREPARATION OF MALTED CEREALS

the specification of which (check one)

- (X) is attached hereto.
 () was filed by an authorized person on my behalf on _____ as Application Serial No. _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and so identified, and I have also identified below any foreign application for patent or inventor's certificate on this invention filed by me or my legal representatives or assigns and having a filing date before that of the application on which priority is claimed.

<u>Number</u>	<u>Country</u>	<u>Day/Month/Year Filed</u>	<u>Priority Claimed (Yes or No)</u>
PCT/BE96/00077 designating United States		July 23, 1996	Yes

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application or PCT international application designating the United States in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

<u>Application Serial No.</u>	<u>Filing Date</u>	<u>Status</u>
PCT/BE96/00077 designating United States Declaration 1194-1	July 23, 1996	Pending

45220-96/00077

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorneys, with full power of substitution and revocation, to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith and request that all correspondence and telephone calls in respect to this application be directed to FITCH, EVEN, TABIN & FLANNERY, Suite 900, 135 South LaSalle Street, Chicago, Illinois 60603, Telephone No. (312) 372-7842:

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Declaration 1194-3

DECLARATION 1194-3